BRNO UNIVERSITY OF TECHNOLOGY

Faculty of Chemistry

BACHELOR'S THESIS

Brno, 2018 Petra Waclawiková

BRNO UNIVERSITY OF TECHNOLOGY
VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ

FACULTY OF CHEMISTRY
FAKULTA CHEMICKÁ

INSTITUTE OF MATERIALS SCIENCE
ÚSTAV CHEMIE MATERIÁLŮ

SPRAYABLE ELASTIC BIOPOLYMER HYDROGELS FOR WOUND HEALING
SPREJOVATELNÉ ELASTICKÉ BIOPOLYMERNÍ HYDROGELY PRO HOJENÍ RAN

BACHELOR'S THESIS
BAKALÁŘSKÁ PRÁCE

AUTHOR — **Petra Waclawiková**
AUTOR PRÁCE

SUPERVISOR — **doc. Ing. Lucy Vojtová, Ph.D.**
VEDOUCÍ PRÁCE

BRNO 2018

VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ
FAKULTA CHEMICKÁ

Zadání bakalářské práce

Číslo práce: FCH-BAK1260/2017

Ústav: Ústav chemie materiálů

Studentka: **Petra Waclawiková**

Studijní program: Chemie a chemické technologie

Studijní obor: Chemie, technologie a vlastnosti materiálů

Vedoucí práce: **doc. Ing. Lucy Vojtová, Ph.D.**

Akademický rok: 2017/18

Název bakalářské práce:

Sprejovatelné elastické biopolymerní hydrogely pro hojení ran

Zadání bakalářské práce:

1. Lit. rešerše na téma elastické hydrogely připravitelné sprejováním
2. Výběr a modifikace vhodných biopolymerů a aditiv pro hojení ran
3. Příprava hydrogelů sprejováním
4. Charakterizace chemicko–fyzikálních vlastností
5. Vyhodnocení, diskuze, závěr

Termín odevzdání bakalářské práce: 29.5.2018

Bakalářská práce se odevzdává v děkanem stanoveném počtu exemplářů na sekretariát ústavu. Toto zadání je součástí bakalářské práce.

Petra Waclawiková	doc. Ing. Lucy Vojtová, Ph.D.	prof. RNDr. Josef Jančář, CSc.
student(ka)	vedoucí práce	vedoucí ústavu

prof. Ing. Martin Weiter, Ph.D.
děkan

V Brně dne 31. 1. 2018

ABSTRACT

The main aim of the presented bachelor thesis is focused on the preparation and characterization of sprayable hydrogels from suitable non-toxic substances, in order to develop gels applicable in wound healing management.

The theoretical part deals with general properties of hydrogels, methods of their crosslinking and presents selected natural and synthetic materials used for the preparation of hydrogels. It also contains a brief overview of already prepared sprayable hydrogels, which are not yet commercially used.

The experimental part describes the selection and use of readily available non-toxic biomaterials and the measurement of the physico-chemical properties of the prepared gels. Gum Karaya, poly(vinyl alcohol) and sodium alginate were selected as the structural polymers together with different physical crosslinkers and additives. The polymers and additives were subjected to solubility test followed by mixing in certain ratios and crosslinked to form preferably transparent hydrogels. Subsequently their optical and rheological properties were valuated.

The measurement of optical properties shows that probably gum Karaya affected increasing in both yellowness as well as transparency. By rheological measuring it was demonstrated, that the higher content of gum Karaya reduces the mechanical resistance of the prepared hydrogels. Hydrogel strength reflects both low and high amount of crosslinker where the optimal amount gave the best result. Therefore, the correct ratio of the used substances is the main parameter for obtaining the most effective hydrogel properties suitable for sprayable hydrogel dressings.

KEY WORDS

Spreyable hydrogels, gum Karaya, transparency, thixotropy, wound healing management.

ABSTRAKT

Cíle prezentované bakalářské práce jsou zamšřeny na přípravu a charakteristiku sprejovatelných hydrogelů z vhodných netoxických látek, za účelem vyvinutí gelů aplikovatelných pro řízené hojení ran.

Teoretická část se zabývá obecnými vlastnostmi hydrogelů, způsoby jejich síťování, a uvádí vybrané přírodní a syntetické materiály používané pro přípravu hydrogelů. Obsahuje také krátký přehled již připravených sprejovatelných hydrogelů, které ale zatím nejsou komerčně využívány.

Experimentální část popisuje výběr a použití lehce dostupných netoxických materiálů a měření chemicko-fyzikálních vlastností z nich připravených gelů. Jako strukturní polymery byly zvoleny gum Karaya, poly(vinyl alcohol) a alginát sodný společně s různými fyzikálními síťovadly a aditivy. Polymery včetně aditiv byly podrobeny zkouškám rozpustnosti, a v určitých poměrech byly smíchány a následně síťovány za tvorby přednostně transparentních hydrogelů. Poté byly vyhodnoceny jejich optické a reologické vlastnosti.

Z měření optických vlastností vyplývá, že gum Karaya ovlivňuje růst jak žlutosti, tak i transparentnosti připravených hydrogelů. Reologické měření prokázalo že vyšší obsah polysacharidu gum Karaya snižuje mechanickou odolnost připravených hydrogelů. Tuhost hydrogelů byla ovlivněna jak nízkou tak i vysokou koncentrací síťovadla, kde optimální množství vykázalo nejlepší výsledky. Správný poměr a dávkování použitých látek jsou tedy hlavními parametry pro získání nejvhodnějších vlastností hydrogelů pro sprejovatelné obvazy na hojení ran.

KLÍČOVÁ SLOVA

Sprejovatelné hydrogely, gum Karaya, transparentnost, tixotropie, řízení hojení ran.

WACLAWIKOVÁ, P. Sprejovatelné elastické biopolymerní hydrogely pro hojení ran. Brno: Vysoké učení technické v Brně, Fakulta chemická, 2018. XY s. Vedoucí bakalářské práce doc. Ing. Lucy Vojtová, Ph.D..

Prohlášení:

Prohlašuji, že jsem bakalářskou práci vypracovala samostatně a všechny použité literární zdroje jsem správně a plně citovala. Z hlediska obsahu je bakalářská práce majetkem Fakulty chemické VUT v Brně a může být využita ke komerčním účelům jen se souhlasem vedoucího bakalářské práce a děkana FCH VUT.

.....
podpis autora

Declaration:

I declare that my bachelor thesis was worked up independently and that used references are correctly and fully quoted. The content of above mentioned thesis is considered a property of BUT Faculty of chemistry and can be used for commercial purposes only with the supervisor's and dean's contents.

.....
autor's autograph

Acknowledgement

I would like to thank my supervisor Assoc. Prof Lucy Vojtova, Ph.D. for her professional advice, graciousness, patience and the whole time spend with my work. I also thank Bc. Kristýna Valová for the rheology measurement and introduction to this issue. I would like to thank all my colleagues from the laboratory for friendly atmosphere and advices. I express my thanks to my family and my friends for their support during the study.

This work was supported by the CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II.

TABLE OF CONTENT

1. INTRODUCTION

Hydrogels were an important discovery in the 60s of the 20th century, when they were used as eyeglasses replacement. Lately, hydrogels became very popular and the number of hydrogel development patents rapidly grew. Actually, the investigation is specialized mainly for tissue engineering, wound healing and burns, where gels are enriched with medicinal substances such as nanoparticles or various enzymes. Because of their soft, supple structure, and tissue-like character, they can easily mimic tissues and adapt to the surface of the wound. The hydrogel layer forms a barrier and, thanks to the high water content, it moistens the wound. Most hydrogels are transparent or slightly cloudy, which make them transparent allowing for monitoring of wound healing. Hydrogel thin films disadvantage is application, while the shape of the film must be modified to cover the irregular shape of the wound perfectly. This problem could be solved by forming a sprayable hydrogel solution, which is able to crosslink in situ on human body immediately after spraying, and thus form a bandage of eligible shape. Synthetic materials used to this day are not enough biocompatible and supporting healing as natural ones, but their usage is predominant due to high prices of natural proteins and enzymes. Natural polysaccharides are biodegradable and relatively inexpensive, therefore by usage of a suitable crosslinking agent, they could form a matrix of hydrogels. The main aim of the work is to prepare cheap sprayable hydrogel, with healing and antibacterial properties.

In this work, polysaccharide gum Karaya is mixed with poly(vinyl alcohol) and borax, which quickly crosslink via gel network forming. The correct ratio of these starting materials are investigated and the optical and rheological properties are measured by a suitable methods.

The first part of the thesis presents a theoretical introduction to the subject, which deals with the characteristics of hydrogels, ways of crosslinking and several materials suitable for the sprayable hydrogels preparation. The next part is focused on the optical and rheological analysis of the prepared material.

2. THEORETICAL PART

2.1 Hydrogels

Hydrogels are natural or synthetic crosslinked polymer networks, which are greatly swollen with water. Combination of natural and synthetic material can produces effective, flexible, mechanically strong, biocompatible and economically prosperous hydrogel dressings. Blends of both represent new materials with better properties than those of the single constituent [1, 2]. 3D network is formed by polymer molecules, crosslinked by chemical or physical bonds. This forming process is called gelation. Hydrogel differs from classical polymer network according to presence of solvent, which coats solid particles of polymer [3]. Despite that, gels are not liquid, they have colloidal character and under lower tensions are inconsistently deformable and elastic. The hydrogel dissolution is blocked by presence of covalent bonds between polymer chains, hydrophobic or electrostatic interaction. [4]. Apart from solution or films, the hydrogels can be also processed into foams, which can be used in preparation of biocompatible aero and xerogels. Hydrogels are useful in separation process, drug delivery, food industry, biomedical, agriculture and cosmetics, according to good chemical and physical properties like adsorption and swelling [5].

Hydrogel structure

According to interconnected pores, hydrogels have a high specific surface, which makes functional groups accessible for ligand fastening and defends against diffusion problems caused by sorption and desorption of material [6]. This mean that hydrogels could be prepared as a stable matrix for drugs carrying. An anionic hydrogel has negatively charged groups, which attract cations (for example COO^-) and anions from a given environment are attracted by cationic hydrogel [7]. For instance, alginate is bonding with Ca^{2+} cations to forming hydrogel structure [8]. If the polymers for gel preparation are not contain reactive functional groups, it is possibility to format these groups on the polymer by post- polymerization reaction [9].

Formed stable matrix is homogenous, hydrophilic and includes two phase. A solid phase is formed of a long chain natural or synthetic polysaccharide possibly combined with synthetic polymer, liquid phase is consisted of water, hydric alcohol, carbohydrates with possibility to mixture with solution of proteins. The matrix is filled with medicament, which is unclenched on the affected areas for local or systemic healing. Crosslinked polysaccharides are plasticized with water and hydric alcohol, which dries very slowly and is dimensionally stable in the water environment. Matrix can be plasticizing by solutions or emulsions of saccharides or polysaccharides and proteins. Finally, hydrophilic substance in matrix moisturizes the skin and improves absorption of a medicament into the skin, by building a hydrophilic bridge [10].

Water in hydrogel

Hydrogel swelling in water depends on the association, dissociation and binding of various ions to polymer chains. Water sorption by hydrogels include hydration due to presence of chemical groups as -OH, -COOH, $-CONH_2$, -CONH,$-SO_3H$ and due to existence

of capillary areas and differences in osmotic pressure. Hydrogels can de-swell according to variations of their outer ambient. These changes can be raised by changing the ambient pH, temperature, ionic strength and electro stimulus [1].

The functioning of hydrogels in various aspects can be considerably affected by different thermodynamic and mechanical behaviours of water. Concerning to water absorption by dry hydrogel, the first water particles entering the matrix will hydrate the most polar, hydrophilic groups following to "primary bound water". The network begins swell and after hydration of all polar groups hydrophobic groups are also interacted by water molecules, leading to "secondary bound water". Combination of primary and secondary bound water is called the "total bound water". Due to the osmotic driving force, hydrogel will absorb additional water after the hydrophilic and hydrophobic parts have interacted with bound water. The contradiction between additional swelling and the chemical or physical crosslinks, has an effect on an elastic network force and causes the equilibrium swelling level of hydrogel. The gel could dissolves by swelling process, if the crosslinks are degradable. According to the bound water, hydrogel may never be totally dried, especially in tissue engineering [11].

2.2 Crosslinking

Crosslinking process of polymers can be done by a chemical reaction or physical reaction. Chemical crosslinking of the chains is aroused by certain reagents added to the aqueous solution [12], while process as a high energy irradiation, enzymatic reaction, radical polymerization or chemical reaction of complementary groups were running. Physical gel chains hold together according to ionic or protein interaction, hydrogen bond or crystallization [13]. Chemical hydrogels is dissimilar to physical hydrogels, because theirs polymer chains are crosslinked by covalent bonds, while the chains in physical gels are only physically crosslinked as shown in Figure 1 [14].

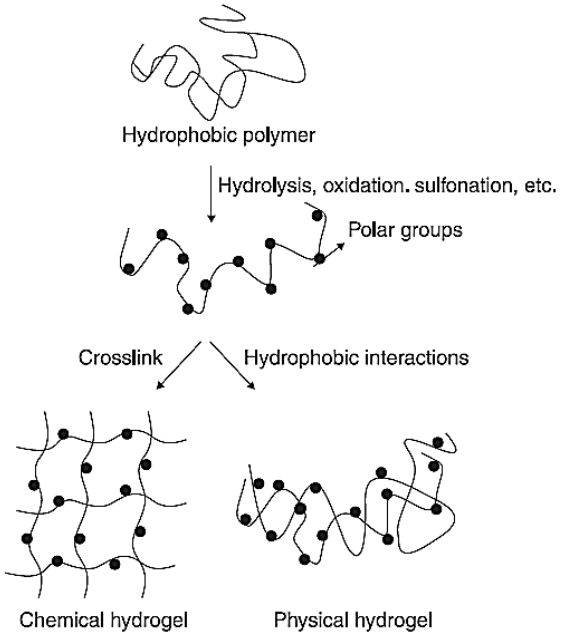

Figure 1: Schematic depiction of chemical and physical gel differences [15].

The degradation of the hydrogel and diffusion drug release are influenced by concentration of the polymers. That is often limited by the gel solubility or high viscosity of the solutions. The concentration also determines the rate of in situ crosslinking and can be increased by lower molecular weight gel, according to needs in different application. Slower-gelling material can give the drugs in pre-polymers time to penetrate the tissues and improve bioadhesion, conversely fast-gelling material may be favourable in surgical setting for ensnare drugs more easily to delay release into body. The amount of added molecule of crosslinker and the density of reactive functional groups on the chains can control the crosslinking density in the hydrogels. Hydrogel formed with higher crosslinking densities are consist of network with smaller mesh sizes, whereby the release of entrapped drugs is reduced. Crosslinking density also determines the mechanical strength of the gel [16].

The synthesis of hydrogel are available to assort into three main categories, which are shown schematically in Figure 2:

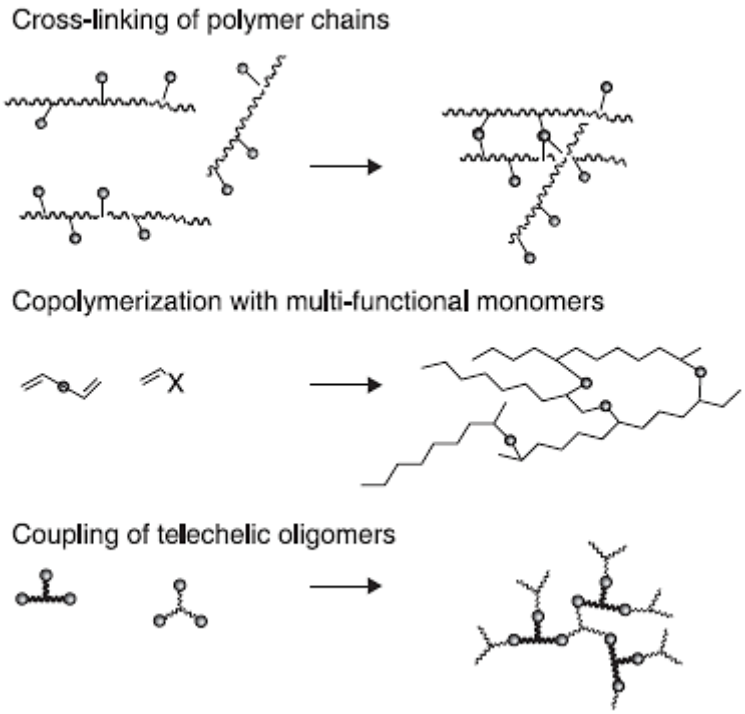

Figure 2: Schematic illustration of the general routes to crosslinked [9].

The choice of a synthesis method depends on the available chemistry and the target application. High molecular weight polymers crosslinking requires either melt processing techniques such injection moulding. Overleaf the telechelic oligomers or the monomer mixtures are capable for solventless and low temperature treatment, according to lower viscosity of their fluids. Multi-functional monomers copolymerize to networking at relatively low conversion. The radical polymerization of water soluble vinyl monomers in the presence of crosslinkers (multi-functional alkenes) is the most commercially used system. Thus prepared hydrogels have highly heterogeneous structures at the molecular level but these materials are frequently used in many application [9].

2.2.1 *Physical gels*

Physical hydrogels are reversible and not homogeneous, since free chain nooses or chain end can create network defect in form of inhomogeneities [11]. These gels are thermo-reversible, whereas chemical gel reaction proceeds on the temperature range. Thermo-reversible gels are used in food and pharmaceutical industry, since the gels are supposed to melt after body entrance in the mouth or in the stomach [12]. The demand of physical gels has been potentially increased last time [17], according to effort avoiding traditional chemical crosslinking agents, which could be toxic. Though they can be isolated from gels before application, they can influence the disposition of the substances like drugs or cells, when they are trapped. Accordingly, the physical crosslinking is preferred for majority of crosslinked gel preparation [18]. Reversible physical gel is possible to dissolve by changing environmental state consist of temperature, pH or the ionic strength of solution. In comparison with it, permanent hydrogels can be crosslinked in the dry state or in solution [15].
Physical crosslinking can be attained by using a species of physicochemical interactions:

Hydrophobic interactions

Crosslinking in water solution via reverse thermal gelation is possible according to presence of hydrophobic domains on polymer chains. These substances are called gelators and they are often slightly hydrophobic. The hydrophobic segment is connected with a hydrophilic polymer segment leading to create a polymer amphiphile, which is water soluble at low temperature. Though, according to the temperature increasing, hydrophobic zones aggregate to minimize the hydrophobic surface area contacting the volume water, in order to reduce the amount of structured water in ambient of the hydrophobic domains and maximizing the solvent entropy. The temperature at which gelation happen rely on the chemical structure of the polymer, its concentration and the length of the hydrophobic block. The more hydrophobic is the segment, the more the entropy of water structuring rise and diving force for aggregation of hydrophobic segments is larger, then gelation starts at the lower temperature [16].

Charge interactions

These interactions were broadly examined for gel in situ crosslinking and can be used to nanoparticle gels, which provide suitable drug delivery properties. Another benefits of this interaction is, that ionic parts in ECF (extracellular fluid) could coupling with the gel components, according to gel network breaks down. Gelation can also be induced by pH changes, which protonate the ionic functional groups [16].

Hydrogen bonding interactions

This phenomenon is often a result of hydrogen-bonding interactions between the polymer chains. Nevertheless, this bonds networks can melt in vivo due to amount of water in short time, unless another crosslinking method is also added [16]. Hydrogen bonding interactions are used for preparation of hydrogels in vitro by freeze-thawing method, for example poly(vinyl alcohol)-based hydrogels [19].

Stereocomplexation

This forming is connected with synergistic interactions between polymer chains molecules of the same substance with dissimilar stereochemistry. These interaction is relatively limited with amount of used polymer compositions, only small changes in composition of gelators matter can weaken or eliminate bonds [16].

2.2.2 *Covalent (chemical) gels*

Chemical hydrogels are non-reversible and can be produced by condensation and polymerization of multifunctional monomers as well as crosslinking of linear macromolecules by chemicals (chemical crosslinking), photoinitiators (photochemical crosslinking), gamma radiation or accelerated electrons (radiation crosslinking). Gamma radiation could replace the crosslinkers, but is brings radiation hazards. Gamma radiation application for medical hydrogels synthesis, including PVA (poly(vinyl alcohol)) hydrogels, is preferable as it allows eliminating residual substance initiators and other low-molecular impurities in hydrogels [6].

Chemical gels can be prepared by one-step procedures like polymerization, as well as multiple step procedures involving using suitable crosslinking agent or synthesis of polymer molecules with reactive groups with successive networking. The polymer gels can be synthetized with control over network structure, mechanical strength, crosslinking density, biodegradation and other biological properties [20].

Chemical hydrogels are ordinarily prepared in two different process: 3D polymerization of the monomer in the presence of a crosslinking agent (Figure 3), or by direct crosslinking of water-soluble polymers, as shown in Figure 4.

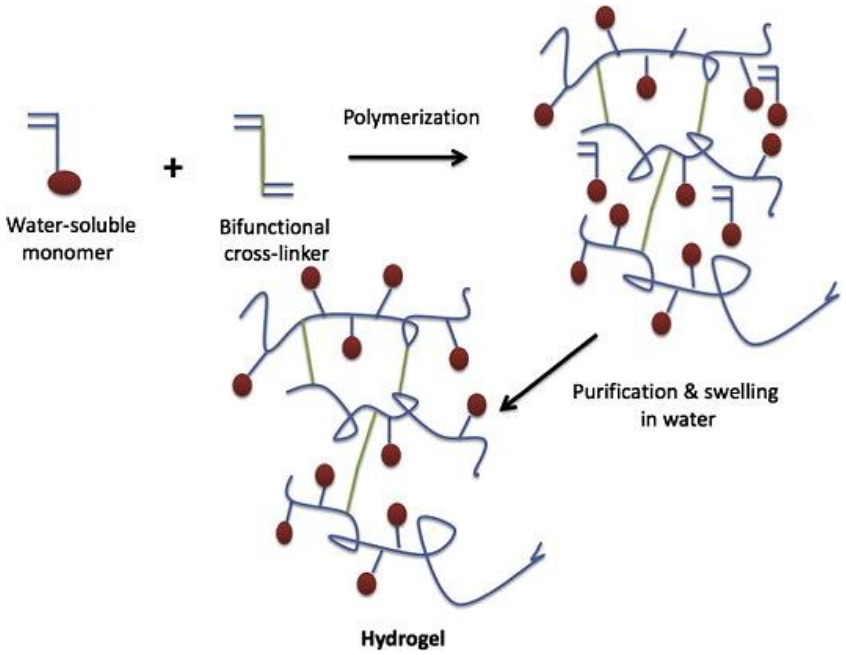

Figure 3: Scheme of three-dimensional polymerization [21].

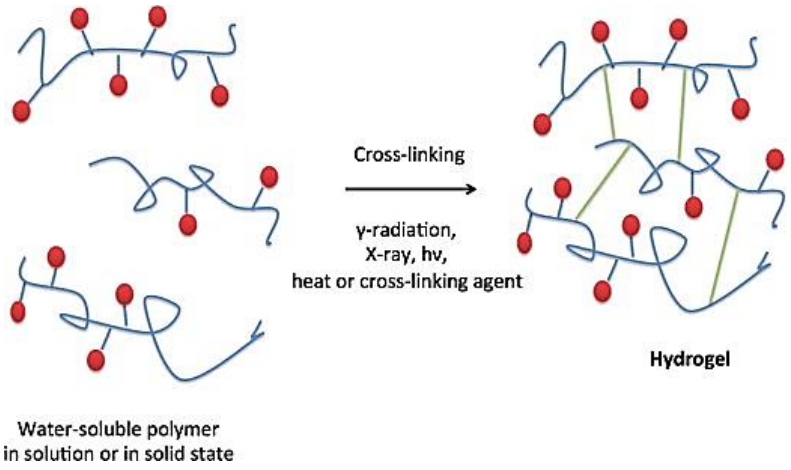

Figure 4: Scheme of crosslinking in presence of crosslinking agent [21].

Small-molecule cross-linking

Small-molecule are used for in situ crosslinking, for instance oxidized sodium alginate is possible to quickly crosslinked with proteins (e.g. gelatine) in the presence of low concentrations of borax to produce hydrogels suitable for drug delivery [22]. It necessary to used it for the drug with two reactive functional groups suitable for reaction of functional groups. This method has considerable disadvantage in the potential toxicity of surplus small-molecule after crosslinking [16].

Polymer–polymer crosslinking

There is considerable advantage of polymer pre-functionalized with functional groups, therefore using of potentially toxic small-molecule like a crosslinking agents is obviated. This method is limited by preparing of the functionalized pre-polymers. Moreover this pre-polymers can be themselves cytotoxic, despite of their synthetize from highly biocompatible substances. This problem is often eliminated by the rapidity of crosslinking, which reduce the amount of unreacted residual polymers. Toxicity could be problematic while the hydrogel degrades to create possible oligomers during reaction with wounded tissue [16].

2.3 Hydrogels and wound healing

Hydrogels content a high amount of water and their mechanical properties make them highly attractive scaffolds for wound healing, for example as nerve prosthesis or for direct injection at the damage tissue for cell growing [23]. The porous structure of hydrogel also provides drugs interception subsequent release. Hydrogels are usually prepared and fill with healing drugs before the placement of the hydrogel on the body. Medications are insert into hydrogel matrices by two ways. In the post-loading method, drug is absorbed into matrix after the hydrogel is formed. Swelling or diffusion force influence the drug realise. In the in-situ forming a drugs are added into a polymer solution before gel networking [24].

Hydrogels can be readily applied to the wound tissue and also lightly removed. Due to their deformability they can modify according to shape of applied surface and some type of hydrogel possibly can be applicate on non-horizontal surface of tissue, due to their

bioadhesive properties [16]. Hydrogels are designed with quality fluid absorption and transparency, which permits to wound healing control [25]. However, disadvantage of hydrogel dressings is their limited permeability to oxygen, which make it unfavourable in struggle against infection. Accordingly, antibacterial colloid compounds added into hydrogel network or density modification of crosslinks in their matrix, are able to increase the porosity of hydrogel [16]. Then during local healing, gel could more transmit the gas and water by using the colloidal materials, which absorb the exudate and form more adhesive gel. These hydrocolloid dressings are easily removable considering small rigidity and they are suitable form thermal isolation and a moist ambient. Impermeable layer provides wound isolation from bacteria and decreases the opportunity of infection. Currently increase inquiry and investigation of dressings, which in addition of wound moisture, will provide drug delivery especially on burns and skin blemish [26]. Most commercially available dressings have a disadvantage in the convenience of application, therefore in situ formed hydrogel will be more practical as dressings [25]. This in situ forms have ability of crosslinking at body temperature, due to their thermoreactivity. Therefore their porous structure provides gas permeability, balance of fluid and water evaporation, moisture and exude (pus) absorption [26]. General advantages and disadvantages of hydrogel are summarized in the Table 1.

Table 1: An overview of hydrogel properties.

Advantages +	Disadvantages −
• easy application and removal	• possible of large pores, which restrict using for a drug delivery
• absorption of exudate from the wound • possible injectability	• dehydration of tissue devoid of proper cover
• add moisture to dry wounds	• gas and oxygen permeability is limited since antibacterial compounds are used
• hydrogel in situ forming	• poor saturability

2.4 Synthetic material for hydrogel

Synthetic hydrogels are used in tissue engineering due to their properties and chemical behaviour. Synthetic polymers can be repeatedly prepared with define structure, molecular weights, block structures, degradable bonds and crosslinking form. These properties influence hydrogel behaviour, mechanical properties and density of 3D network [27].

Lately, synthetic hydrogels substitute the natural hydrogels due to strength, large water absorption, long liveness of synthetic hydrogel [4]. They have great flexibility in synthesis and modification, though they have problem to recognize surface cell according to deficient cell affinity [28]. During polymer-polymer crosslinking the gel may contains residual unreacted crosslinking agent or unreacted residual polymer, so it is necessary purify the hydrogel. The possibility of avoiding gel purification is using synthesis by crosslinking prepared polymers, which are soluble in water such as polyacrylamide, poly(ethylene glycol), poly(acrylic acid) or PVA (poly(vinyl alcohol). These polymers are generally used in biomedical and pharmaceutical industry due to their nontoxicity and elimination of purification [21].

Poly(vinyl alcohol)

Hydroxyl-functional monomers using is the best way how to add hydroxyl functionality on polymer chains. It is material with chemical formula $(C_2H_4O)x$, which commonly contain –OH groups. Chemical structure of PVA is very elementary as well as simplicity of its reaction. This water-soluble polymer has high amount of hydroxyl groups and there are various routes to gel crosslinking [9].

PVA hydrogels are suitable for the preparation of biopolymers due to their good properties such as non-toxicity, non-carcinogeneity, biocompatibility, excellent film-forming, excellent transparency and ability to excellent film-forming. According this advantages, poly(vinyl-alcohol) is capable of natural tissues imitation and is easily accepted by the human tissue. Hydrogels prepared from PVA are use in addition to wound healing and drug delivery as contact lenses and it is the oldest synthetic polymer used for hydrogel preparation. According to excellent transparency and smooth surface, this hydrogel is widely used as dressing for burn injuries [21]. Despite the good mechanical properties of PVA gels, their insufficient flexibility is limiting for their use as a self-contained wound healing matrix [29]. Therefore, most commercial PVA hydrogels are synthetized with as mixture with synthetic or natural polysaccharides (such as alginate, chitosan, gelatine, glucan [18]) to conserve biological compatibility [30]. Final properties of the material thus prepared depends on the properties of the separate substances and on the change properties of PVA hydrogel after mixing [31].

Water contained in PVA hydrogel forms a moist environment suitable for rapid wound healing, while facilitating penetration of drugs, nutrients and gases into a localized wound. The disadvantage of the PVA solution is reduction of the strength of the prepared gels while long-stored solution is used. [18].

PVA hydrogel can be synthetized by various type of crosslinking, e.g. reaction with anions or gamma radiation. It was investigated, that using this radiation have positive effect on mechanical and physical PVA hydrogel properties, which are better than properties of gels crosslinked with chemical initiators. The main disadvantage is the exposure of radiation, which could be hazardous for the patient [6].

Borax

Sodium tetraborate $Na_2B_4O_7$ is an inorganic compound, and form one perspective class of hydrogels, while PVA is complexed with (THB) tetrahydroxyborate anions [32]. The complexation reaction is described on Figure 5.

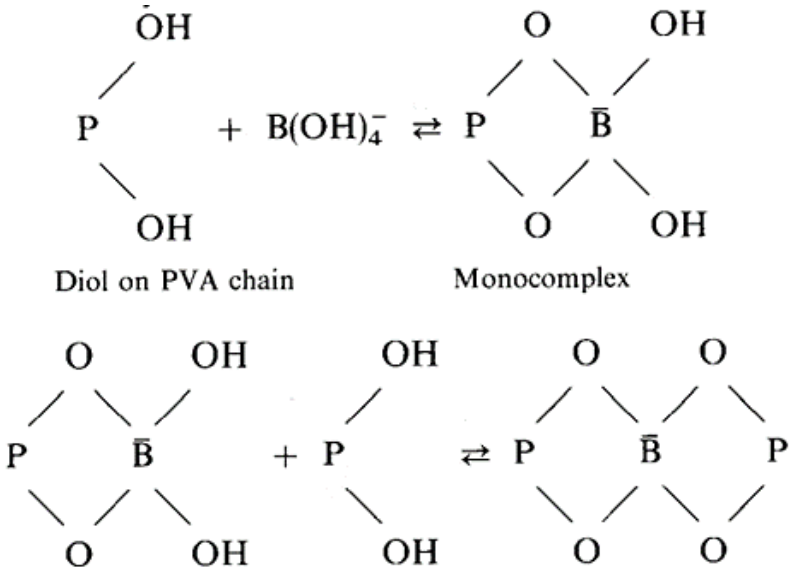

Figure 5: Scheme of complexation reaction of borate ions with PVA [33].

Though gelation of complexing polymers is usually slow process, the formation of the complex passes quickly and reaches the equilibrium in few minutes. The excess of added borate can shrink the homogeneous gel, which causes the segregation and then another added borate ions can again provide a homogeneity of the hydrogel. This reversible ability depends on many parameters, which are difficult to controlled or adjusted [33].

The phase behaviour of the sol-gel transition of aqueous PVA solutions with borate ions can be investigate in more detail. PVA molecules form charged complexes with borate ions, which are obtained from borax solution or a mixture of boric acid with sodium hydroxide. The viscosity of these complexes is a function of concentrations of borax or boric acid. At the beginning of gel synthesis the intrinsic viscosity η decreased and then increased with higher concentration of borate ions in solution. Thermo-reversible transition can be achieved without phase demixing according to increasing concentration of polymer. The gelation concentration achieved the similar value as concentration of overlapped chains which respond to η. All these facts correspond to existence of intra- and inter-chain crosslinks and electrostatic potentials, which guarantee a balance of the excluded volume effect [34]. The detailed mechanism was investigated by magnetic resonance in 1986 in the study [35].

A recent study from 2012 [32] describes, that the THB anion can interact with two different cis-diol groups on PVA, which loads to the formation of a di-diol complex. The study also describes, that effect of the THB concentration is larger than concentration of PVA. This is explained by the fact that, after dissociation of the borax, produced sodium ions support the second step of the complexing di-diol reaction, according to crosslinking density increases [33]. Therefore, rheological properties of this hydrogels depend as on initial concentration of borax, as on concentration of poly(vinyl alcohol). Change of concentration influence compressibility, hardness and adhesiveness of completed gels. More than the concentration of PVA, the gel stiffness is determined by its structure properties. The prepared hydrogel will be more rigid while using a polymer with a higher molecular weight and higher degree of hydrolysis, which reduce the number of released free polymeric materials by reducing

diffusivity and increasing network density. Increasing the PVA concentration surprisingly causes an increase of the crossover frequency even though the THB concentration rests low.

This is due to the displacement of the equilibrium of the reaction in behalf of the mono-diol reaction, because the increased concentration of PVA decreases the concentration of di-diol crosslinks. Temperature also determines the number of PVA-THB bonds. Increasing temperature causes smaller mechanically rigid of gels due reducing of network formation.
The adhesiveness, biocompatibility and other properties of PVA–THB hydrogels made them a challenge for the deeper investigation in wound engineering [32]. In general, the low concentrations of used borax have the favourable effect of the solubility increasing and compatibility of biopolymers due to complexation [22].

Borax can accelerates another gelation of polymers, for example mixture of nature polysaccharides alginate and gelatine, to rapidly hydrogels forming [22].These biodegradable and non-toxic hydrogels are potential as in situ forming matrices for drug delivery. Borax solution has been used in medicine for many years now as an eye-drop, mouth wash and skin cosmetics, due to its antiseptic and antiviral effect [25]. In Hunt's study [36] is descripted how boron helps in inflammation healing via decreasing of enzymatic activity during inflammatory process.

Glycerol

Propane-1,2,3-triol is hygroscopic, colourless viscous and odourless liquid. Glycerol is a non-toxic monomer with good properties for hydrogel synthesis, such as biocompatibility, biodegradability and hydrophilicity [37]. According this is commercially used as lubricant, water evaporation retarder and also increases hydrogel swelling ability [39]. Using glycerol in biopolymer gels would also provide decreasing of the pressure on the water resources and minimize environmental pollution [40]. Glycerol molecule contains three -OH groups, therefore it has a great water-affinity, so that this alcohol is commercially used in food additives, cosmetic and medical branch as surfactants and plasticizer [41].

Plasticizers are added to pharmaceutical films, because of their rigidity and fragility after moisture loosing. They also can improve mechanical properties, elasticity and resistance of spray bondage and facilitate their application by flow improve. Glycerol belongs to hydrophilic plasticizer group as same as propylene glycol, glycerine triacetate or oleil-oleate [42].

Due to presence of hydroxyl groups is glycerol able to crosslink with some natural polysaccharides through hydrogen bonds forming. For example cassia gum-glycerol based hydrogel films have high tensile strength according to increasing glycerol concentration [43].

2.5 Nature material for hydrogel

Natural polymers for hydrogels preparation are important material due to renewable resources attainment, several polysaccharides are available from plant stocks. All of them have high amount of hydroxyl function groups and there are more ways to crosslink them [9]. Biocompatibility, biodegradability, and biologically recognizable groups which support activity of cells are substantial advantages of natural hydrogel, through their mechanical properties are not as notable as synthetic material features. Moreover, natural polysaccharides

can contain pathogens or induce inflammation [1]. If these phenomena do not occur, natural hydrogels are suitable materials for wound dressings due their wound moist providing. According to their transparency the wound healing process can be easily controlled. Various shape and size of prepared hydrogels films make them permeable to O_2 and CO_2 though the passage of microorganism reducing [44,45].

Polymer properties are able to be modified by several methods, e.g. by widely used grafting [2]. The natural polymers are more suitable for the grafting than the synthetic polymers due to their low price, easy availability, non-toxicity and biodegradability. It is also possible to functionally modify the natural polymer chains, for example gum Karaya can be modified by polyacrylamide to form pH-sensitive spray dried microspheres for anti-cancer drug [46].

Gelatine

Gelatine is an amphoteric natural polymer, which forms thermally-revisable and high mechanical hydrogels, films, hydrogels matrix, viscous polymer solution, but its drawback is fast biodegradation and lower thermal stability after heating. Gelatine (GE) is a protein produced by denaturalization of collagen, which is commonly extracted from the bones, organs, connective tissues and bowel of domesticated animals (e.g porcine, horses) [23].

GE has several advantages as renewability, non-toxicity, low cost, biocompatibility and high-water absorption and biodegradability. It also has gelling properties due to the presence of a large amount of functional groups, such as amino, carboxyl or hydroxyl, which load to chemical or crosslinking [47]. Gelatinization ability is also provided by certain surface activity when gelatine is able to form hydrogen bonds, electrostatic and hydrophobic interactions by physical crosslink [48]. Obliviously, physical gelatine hydrogels are prepared by cooling solution below the gelation temperature, which was pre-heated to dissolve it, due to gelatine temperature-depended behaviour [12]. Gelatine's biological activities makes it suitable for use in wound dressing, tissue engineering and drug delivery systems [23].

Chitosan

Chitosan is important poly(D-glucosamine), produced by deacetylation of chitin and used for the hydrogels synthesis [9]. Chitosan's scaffolds properties can be improved by chemical or enzymatic modification, while there is various types of scaffold such as gel, film or particulate systems [49]. Chitosan has cationic amine groups, which make this polymer suitable for cell adhesion support [50]. Molecular weight and the degree of deacetylation determines chitosan electrostatic or physicochemical properties such as solubility and chain conformation [51,52]. Chitosan scaffolds are biocompatible, non-toxic and mechanically stable, while the properties can be enhanced by combination with other polymers. These systems are suitable material for soft tissue engineering due to their porous structure, high affinity to in vivo macromolecules and mucoadhesivity [49]. Chitosan is preferred over chitin in common application via its solubility in acidic, neutral and alkaline solutions. The problem of chitosan is its high viscosity, which limits its application. This problem is resolved by the chitosan's chains hydrolysis in alkali ambience by the molecular weight decreasing. However, lot of additional studies are needed to improve this material using not only in laboratory but also in commercially clinical application [51].

Xanthan

Xanthan gum high molecular weight acidic polysaccharide mainly produced by bacterium *Xanthomonas campestris* [43]. It is commonly used as thickener agent in drilling fluids, cosmetics and food product. As same as carrageenan, xanthan is able to be chemically crosslinked via non-toxic citric acid [14]. Xanthan's backbone is consisted of several five sugars. Xanthan's monomer has up to three carboxyl residues at its side chain (from glucuronic acid, acetyl and pyruvate residue), which makes the structure of xanthan more complex and provides a variety of xanthan types. They have different chemical and physical properties due to the different concentration of acetate and pyruvate residues in the monomer [5].

Alginate

Alginate is hydrophilic, biodegradable, biocompatible, and relatively economical natural polysaccharide. It is used in medical application such as wound dressings or surgical or dental impression material, though the skin allergic reaction has been recorded in two studies [54]. Alginate's using is widely due to its gelling properties in aqueous solutions, while alginic acid gel can be obtain by the interactions between the carboxylic acid moieties and bivalent ions, (such as copper, lead or calcium) or by lowering the ambient pH [30]. Alginate is suitable for in situ injection, crosslinking is under very mild conditions, but its disadvantage is mechanical weakness and mainly difficult handling, storage is commonly in solution [54,55].

Alginate is produced by bacteria, but is commonly extracted from marine brown algae. It is composite of a β-d-mannuronic acid (M) and α-l-guluronic acid (G) (Figure 6) and their ratio regulate hydrogel elasticity [56]. Alginate can be physically crosslinked by calcium ions (Figure 7), which prefer networking in the guluronic segments. Their structures have significant effect on the matric forming [57]. Calcium ions is also bioactive in several biological process, therefore is suitable for hydrogel wound healing application [58].

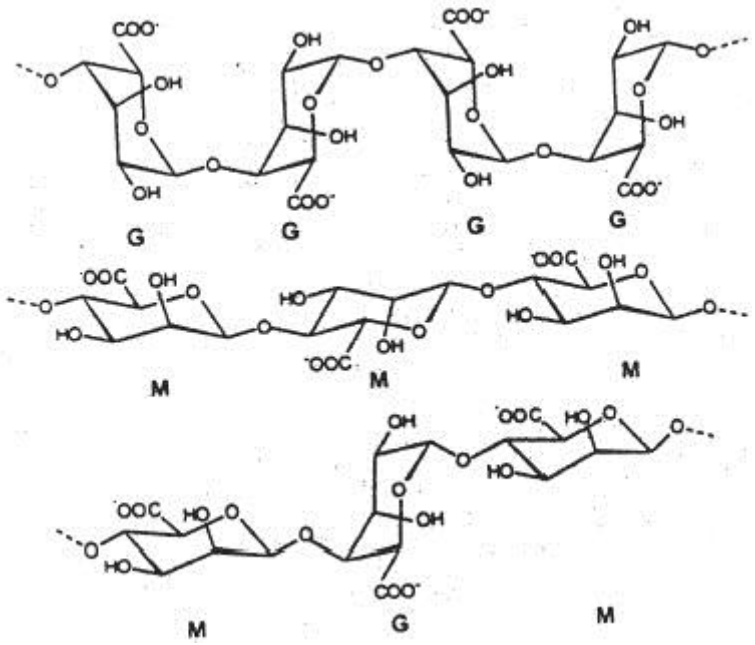

Figure 6: Scheme of alginate block types: G = guluronic acid, M = mannuronic acid [8].

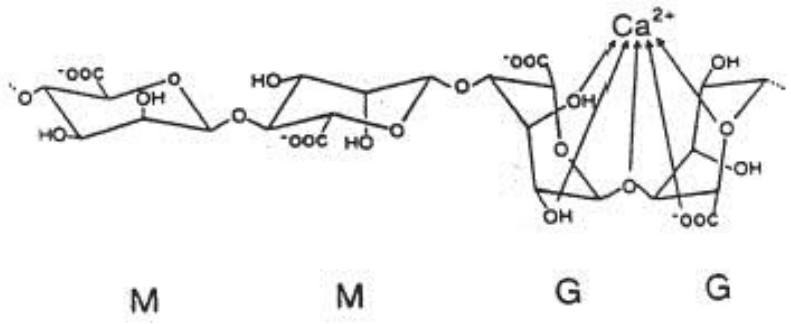

Figure 7: Scheme of probable bonding between the calcium ion and two G residues [8].

Sodium alginate (SA) has been investigated for wound dressing application combining with other material, such as PVA, borax or gelatine, to form the dressing structure which moisten wound ambient due alginate high water swelling ability [18]. Increasing of alginate oxidation reduces the gelling time of combined hydrogel, which was investigated in recent study [22], where alginate dialdehdyde (oxidized alginate) was mixed with borax and gelatine to form hydrogel for medical usage. However, alginate gels degradation is uncontrollable, they release high molecular weight fibres, whose removal from human body can be difficult [59]. Non-toxicity of these SA-borax-gelatine hydrogels were proved by cytotoxicity screening, so they have potential in drug delivery, burn dressing and tissue engineering [22]. Apparently, used borax has antiseptic effect to reduce bacterial accumulation on the wound. The wound healing ability of these hydrogels can be elevated drugs or growth factors addition [25].

Gum Karaya

Gum Karaya (GK) is considerably branched polysaccharide which intricate composition. Commercially is gum Karaya obtained from *Sterculia urens*, a high soft-wooded tree, growing in India and Pakistan [60]. Therefore, is GK widely available and comparatively cheap. During storage a slight vinegar scent can be noticed, due to acetic acid realising, because the gum is partially acetylated. Gum Karaya swells in 60% alcohol solution, but is insoluble in water and other organic solvents, unlike other natural gums [61].

GK chains contain galactose, rhamnose, galacturonic acid and other substances. Exact structure is difficult to describe due to high molecular weight [62]. GK swells in water due to presence of the acetyl groups, which accurate position on chain is unknown yet. The usage of this biomaterial is limited by insolubility in water, which is worsen by presence of the acetyl groups [63] due to hydrophobic character of the methyl group. Acetyl groups can be removed from GK by hydroxide because they are labile at pH = 12 [64]. This deacetylation process and its effect on solubility is demonstrated in Figure 8.

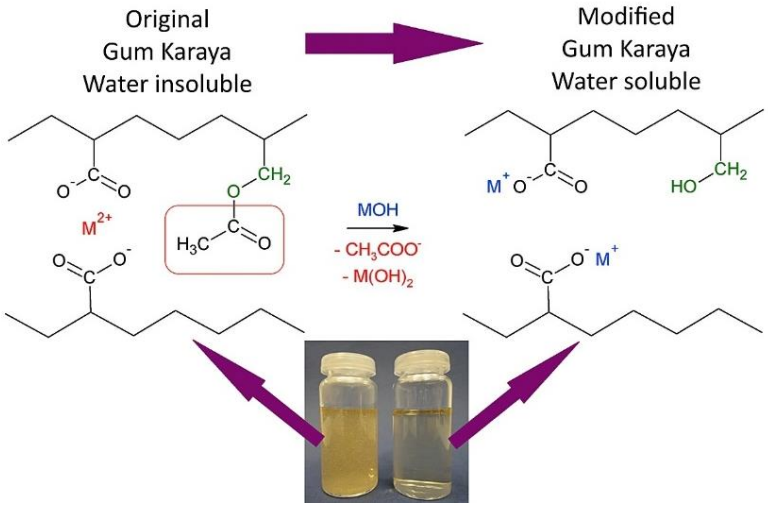

Figure 8: Presumed scheme of GK deacetylation, where M^+ is Na^+, K^+, Li^+ or NH_4^+ and M^{2+} is Ca^{2+} or Mg^{2+} [65].

Bivalent ions can crosslink two anionic groups (e.g. hydroxyl and carboxyl groups) by electrostatic interactions [66], which worsen material solubility in water. Mixing of GK containing –COO groups with excess of a strong hydroxide induce a replacement a part of bivalent ions by monovalent ions [67] which increase the solubility of GK [65].

GK can be mixed with sodium alginate and crosslinked by Ca^{2+} ions due to interaction with COO− groups in both polysaccharides [8] as shown in Figure 9. The calcium cations fit into the electronegative recesses, while the term "Egg Box" model is used, and created crosslinked sections are called connection zones [68].

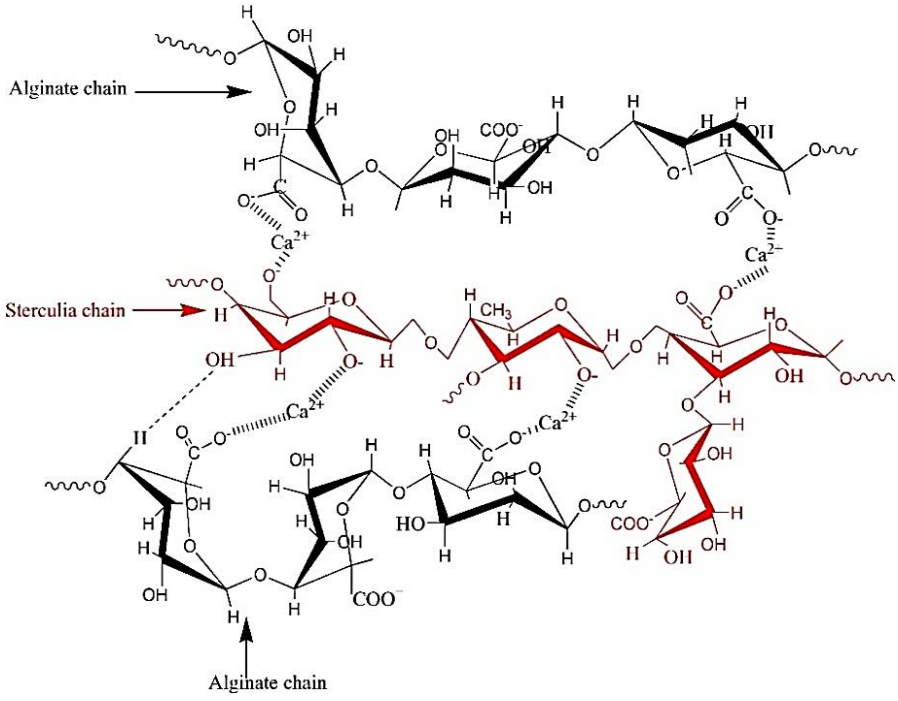

Figure 9: Scheme of interaction between GK, alginate and Ca^{2+} ions [69].

In recent years, combinations of GK with other natural or synthetic polymers, are investigated due to their unique properties, comparatively low price and availability of GK. For example, gum Karaya was modified by PVA and crosslinked to gel forming through gamma radiation, to investigated hydrogels suitable for wound healing and drug delivery matrices [53].

2.6 Sprayable hydrogels

In intention of accessibility of hydrogel application, scientist try to develop spray container filled with polymer solution, which will be able to crosslink in situ after spraying on skin. Some gelatin-based sprayable foam as skin substitute have been prepared in 1950s, but due to bacterial skin coverage it never been reached the biocompatibility of sprayed gel [7]. Lately, some spray bondage were developed and patented (e.g. aerosol spray containing a film-forming hydroxycarboxylic acid polymer in 1993 [48]), but commercialization of these material was still problem for pharmaceutical companies. Since 2000s development of sprayable bandage become intensively investigated. The first bigger applied research developed bandage based on PVA mixed with poly ethylene glycol (gel base), propylene glycol (strength amplifier), triethanolamine (for increasing of viscosity). As plasticizer were added formaldehyde, which that accelerate the drying. Another prepared solution for bandage had problem with stability, toxicity, stickiness, tackiness, solubility and pH dependence. Arun Radhakrishnan et al. [42] worked up comprehensive review of already prepared skin bandage with comments about their advantages and disadvantages.

In 2015 Mohammed H. Mahdi et al. [70] investigated how to modify gellan solution, which formed elastic gels only below 60 °C, therefore it is difficult to use it as sprayable gel. They tried to solve this problem by a shear force applied during gelation process to form fluid gel containing high acyl gellan. Thus prepared gel system contained already gelled micro-particles suspended in a solution of un-gelled polymer. The rheological properties of thus prepared material enable used gellan fluid gel as a standard nasal spray device.

Boon-Beng Lee et al. in 2018 published study [71] deals with spraying of calcium chloride droplets on alginate film towards gelation. The study is useful to determine the gelation of alginate with limited amount of Ca^{2+}, but do not clarify the using of alginate for sprayable hydrogel forming.

Several prepared sprayable hydrogel

This section contains small review elaborated via thorough reading of academic articles, which deals with already prepared sprayable hydrogel. Their preparations and disadvantages are summarize in Table 2.

Relatively new hydrogel spray were introduce by Nasim Annabi et al. in 2017. The composite hydrogel was consisted of two biopolymers from ECM proteins (extracellular matrix), such as gelatin methacryloyl (GelMA) and methacryloyl-substituted recombinant human tropoelastin (MeTro). Additionally, both of them have been were added antimicrobial peptide Tet213.

MeTro/GelMA hydrogel could be photocrosslinked via ultraviolet light, although it’s using is speculative due to possible DNA damage. Therefore visible light-activated photoinitator

system were added into hydrogel for stable biosafety. System was consisted of Eosin Y, TEA (triethanolamine) and co-monomer poly(N-vinylcaprolactam) (VC), which also provided gel elasticity. Process of crosslinking is radical, when Eyosin Y molecules are excited by visible light to triplet state, which abstracts hydrogen atoms from TEA. Reproduced radicals initiate vinyl-bond crosslinking of methacryloyl biopolymers with VC. Mechanism of reaction and gel application is shown in Figure 10.

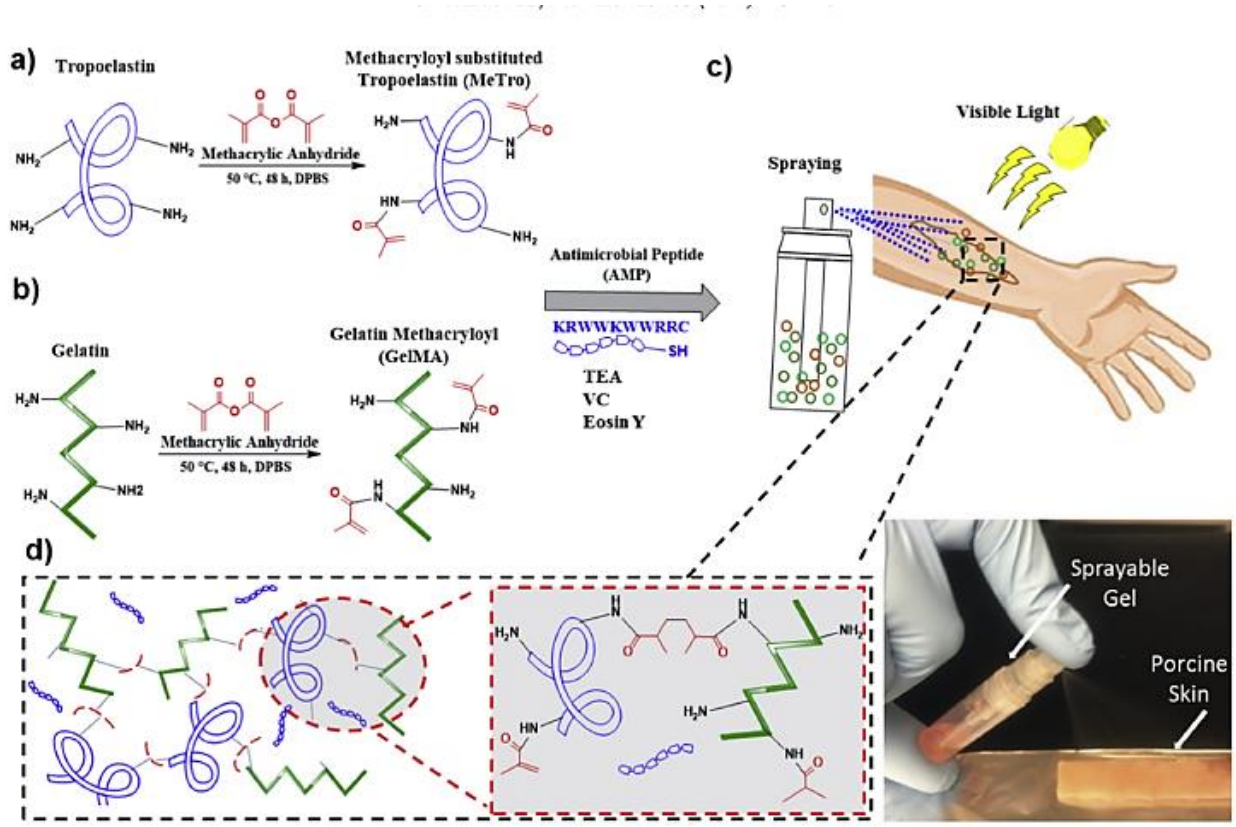

Figure 10: Complex scheme of synthesis MeTro a); synthesis of GelMA b); illustration of light crosslinking and gel application; detailed mechanism of crosslinking d) [134].

In order to maintain biocompatibility, visible light and enzymes were used, which means the high price of the hydrogel. Therefore commercialization of this product will not be simple. In addition, the important properties of hydrogel such as swelling and shear strength are mostly provided by gelatine, and other expensive substances are added only for the purpose of safe health crosslinking [72].

Another sprayable hydrogel invention introduced in 2017 was by Jostein Gripa et al., where the β-Glucan was chosen as an active ingredient and Carbopol 971P NF as the thickening agent in due to its low viscosity, low toxicity and high transparency. Hydrogels have been proved to be long-lasting, but experiments on mice has shown, that carbopol did not support faster wound healing and caused adverse reactions. Therefore, the investigation of this sprayable gel is still open to the development of a better thickening agent than used carbopol [73].

It is also possible to prepare a sprayable hydrogel in a double step spraying process as well as Mr Yoon D. Suk et al. in 2016. In their study [74], they first prepared the conjugate gelatine gel via HPA (3-(4-hydroxyphenyl) propionic acid) activated by enzymes, such as EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) and NHS (N-hydroxysuccinimide). According to wound healing support, gelatine conjugates were combined with two types of chemokines, small signal proteins, which assist in regulation of cell migration. Thus prepared gelatine pre-hydrogel was crosslinking via enzyme-catalyzed oxidative reaction in the presence of HRP (horseradish peroxidase) and H_2O_2 (hydrogen peroxide). The GH (gelatin-hydroxyphenyl-propionic acid) hydrogels were formed by substances mixing at room temperature using spraying method. Important disadvantage of this sprayable hydrogel is transferring into commercial sphere due to the high price of used enzymes and crosslinkers.

Obviously, the greatest potential of the above-mentioned sprayable hydrogels is spray-by-spray in situ crosslinking alginate hydrogels delivering a tea tree oil microemulsion (MeTTO). Tea tree oil, water, polysorbate 80 and ethanol provide stable microemulsion with good antimicrobial effect. Alginate (Alg) solution was sprayed on a lightly wet glass Petri dish (pre-dispersing of MeTTO in Alginate solution), followed by a spray of a $CaCl_2$ aqueous gelling solution and a thin layer of hydrogel was formed immediately. Antimicrobial effect of thus hydrogel increased the potential of the system as using in wound dressing [75].

Oil and water emulsion mixed with anionic polysaccharides probably has high oxidative stability and longer shelf life due to used biocompatible polysaccharides [76].

Table 2: An overview of each sprayable prepared hydrogel.

Materials	Modification	Another substances	Solvent (or stabilizing agent)	crosslinking application (or agent)	advantage or disadvantage	
Gelatine and Tropoelastin	Methacrylation	-Tet213 (antimicrobial peptide) Initiators: -TEA (triethanolamine) -Eosin Y - VC (for elasticity)	water	Visible light (induced as a result of interaction with electromagnetic radiation in the visible and near-visible range.)	— high price + Main source of swelling and shear strength is gelatine	[71]
Beta-1,3/1,6-glucan	Carbopol 971P NF (thickening agent)	NaOH (pH adjusted)	-Water - 1,2,3-propanetriol glycerol	Hydrogen bonds between OH groups	— Only glucan is active healing ingredient	[73]
Solution of gelatine hydrogel conjugates (contain phenol groups)	Chemokine : IL-8 or MIP-3a	Substances for hydrogel conjugates: 3-(4-hydroxyphenyl) propionic acid (HPA) Acctivated by: 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) And N-hydroxysuccinimide (NHS)	-Dulbecco's phosphate buffered saline (DPBS) -HPA solution: Water and dimethylformamide	H_2O_2 + horseradish peroxidase (HRP)	+No effect of used chemokine on hydrogels stiff — high price	[74]
alginic acid sodium salt	Microemulsion of TT oil in water	Polysorbate 80	-Water -ethanol	$CaCl_2$ (calcium chloride) aqueous solution	+Long stability of TToil emulsion	[75]

2.7 Hydrogel characteristic

Most important investigated hydrogels properties are non-toxicity, transparency, elasticity and mechanical endurance and another mechanical property. They commonly depends on the structure, behaviour, origin and crosslinking degree of the used material. Increasing this degree leads to very stiff gel, conversely heating the material reduce hydrogel stiffness. Generally mechanical properties depends on many aspects, for example Young Modulus, which can increases through crosslinking density. This modulus and another properties (such as Poisson modulus, tanδ, storage and loss moduli) can be determined by rheometer. Young Modulus in a hydrogel is affected by water and gel matrix connection [77]

2.7.1 *Rheology*

Rheology is study investigating flow and behaviour of material during and after deformation. It is possible to study all matter including materials with extreme viscosity or elastic module. The behaviour of the material is divided into elastic and viscous. The elastic behaviour is reversible, which means that the external force disappearing, causes the return of the fabric to its previous state. As opposed to, the viscous behaviour prevails, the changed state persists even the external force are removed. Polymers and their melts or solutions exhibit viscoelastic behaviour as they react to external stimuli as elastically as visually. Dominating reaction then determines the overall behaviour of the polymeric material under stress [78].

Rheology is commonly used to characterize non-Newtonian liquids, because its viscosity varies either with time, temperature and mainly external deformation, therefore viscosity is a function of shear rate or shear stress, because their ration is not constant during stressing. This phenomenon is called the apparent viscosity. Non-Newtonian substances exhibit different behaviour under different conditions based on structure and inner interaction. On the contrary, the viscosity of the Newtonian fluid is independent on the external conditions, and is only the function of temperature [79].

For non-Newtonian fluids, there exist dependency between shear rate (γ) and shear stress (τ) where shear rate is function of shear stress. This relationship is graphically shown in Figure 11, where four basic types of time-independent fluids are illustrated.

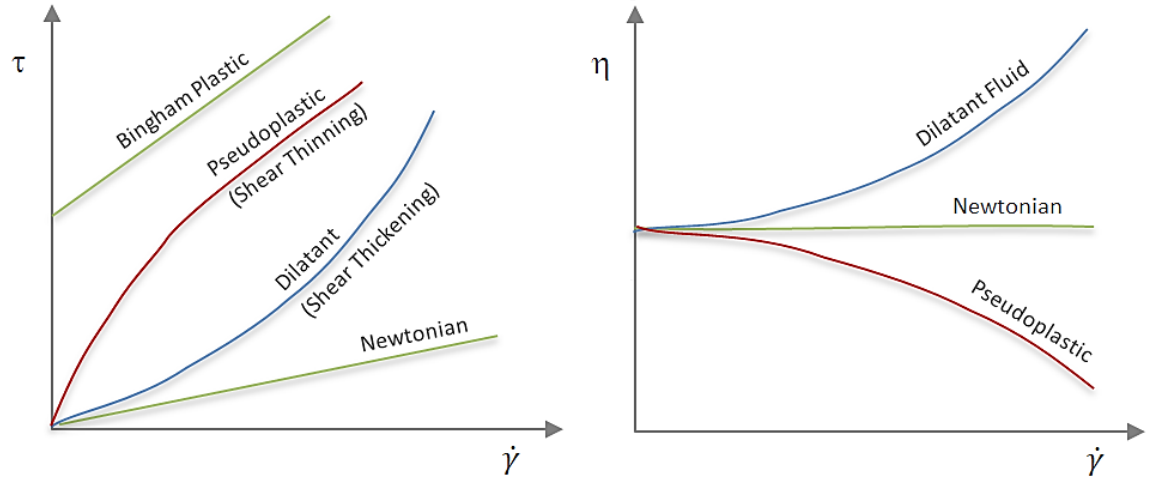

Figure 11: Graph of (γ)= f(τ) function for four basic types of time-independent fluids [80].

These fluid types are idealized real flowing of fluids. Most polymer solutions and melts are pseudoplastic materials, due to shear thinning, while shear-thickening or dilatant behaviour is not so common phenomenon. Bringham plastic fluid are Newtonian, which mean that their viscosity is constant during shear rate increasing as is shown in right side of Figure 9 [81].
In the context of viscosity assessment, the substances behave either reopexiously or thixotropically. The apparent viscosity of thixotropy substances decrease due to long-term shear stress influence [82]. Reopexia is exactly the opposite process when the viscosity increases with the duration of stress. Both of these properties are demonstrated by hysteresis behaviour, which recognized during measuring by a typical hysteresis loop (Figure 12) [**Chyba! Nenalezen zdroj odkazů.**]. Thixotropy behaviour is proved by Steady state testing ethod, while the shear stress is measured during linear shear rate changing [83].

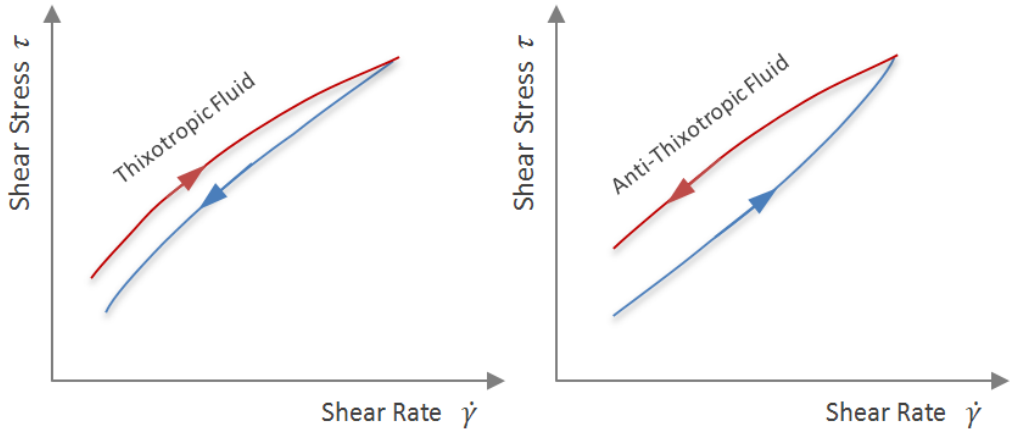

Figure 12: Scheme of hysteresis behaviour of thixo and anti-thixotropic fluids [84].

Rheology measurement

For kinetics of the gelling reaction and the associated stress-dependent changes in viscoelastic properties, is suitable a rotary rheometer AR G2 (Figure 13), which measure the samples by dynamic and steady state analysis.

Figure 13: Used rotary rheometer [147].

It is necessary to select a suitable geometry for viscoelastic measurement, commonly available geometry is cone plate, parallel plate and concentric cylindrical, which is used for very viscous liquids. For hydrogel system, the most used geometry is cone plate or parallel plate geometry [148].
During dynamic oscillatory testing, the phase difference designates the storage (elastic) G´ and loss G´´ (viscoelastic) modulus. It is important to measure the samples in the linear viscoelastic area, due to G´ and G´´ independency on frequency, stress or amplitude of strain [149]. For oscillatory testing several types of sweep are used, their description is summarize in Table 3:

Table 3: Review of type of dynamic rheological testing methods [188].

Testing	**Constant**	**Change**
Strain sweep	Frequency, temperature	Strain
Time sweep	Strain, frequency, temperature	Time
Temperature sweep	Strain, frequency	Temperature
Frequency sweep	Strain, temperature	Frequency

2.7.2 *Optical properties via ultraviolet–visible spectroscopy*

Some natural polysaccharides, plasticizers, solvents or another hydrogel additives can determine the gel colour or transparency, which is important for wound monitoring while healing [28]. It is possible to measure the optical properties of hydrogels by ultraviolet–visible (UV-VIS) spectroscopy, which is commonly used rapid and precise analytical method.

CIE lab colour space

Quantification and expression the colour of items is difficult for human, and therefore has been developed *L*a*b* system, "tristimulus coordinates". These three-dimensional colour models (shown in Figure 14: The CIELAB colour space model [92]) have been developed by the professional scientific organisation CIE (International Commission on Illumination'). Lab colour space quantify and describe the colour via three parameters. These include colour coordinates, points in colour space on horizontal axes, *a** and *b**, where +*a** is the red direction, –*a** is the green direction, +*b** is the yellow direction, and –*b** is the blue direction. Parameter L* symbolize the lightness, ranging from 0-100, and can be used to determine the material's transparency [89]. Lightness indicates how much light energy sample reflects or transmits [90]. Zero is the centre of each axis and describe items colour as neutral, which can be appointed also with very low numbers of a*and b*. These parameters have no maximum defined, but they are commonly numbered from –128 to +127 [91].

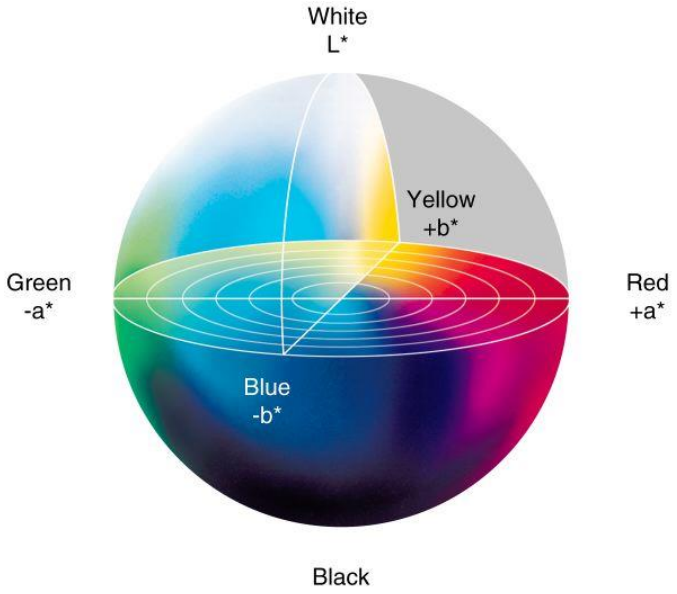

Figure 14: The CIELAB colour space model [92].

Absorbance

Determination of substances by molecular absorption spectrometry is based on the measurement of the absorption of electromagnetic radiation by the molecules of the measured substance in the ultraviolet and visible area (200-800 nm). Substances which absorb radiation with a wavelength less than 380 nm (UV radiation) appear to the human eye as colourless, on the contrary, substances absorbing white light from the wavelength range 380-770 nm, appear to be colour [93]. Organic compounds are relatively transparent in the UV and VIS regions. Several specific types of organic materials absorb both type of radiation, which provide useful information about quantitative analysis or identification of substances [94].

3. AIM OF WORK

The main goal of this work is to prepared novel sprayable hydrogel from available and cheap material, which will be biocompatible to act as a skin dressing and support wound or burn healing. To exclude unsuitable materials and to use the most appropriate ones, the following steps were taken:

- Solubility of original material testing (gelatine, natural and deacetylated GK, benzoic acid, alginate in water, glycerol and ethanol)
- Crosslinking agent stability in solution (borax in water and glycerol)
- Preparation of the material (GK mixing with PVA, glycerol, borax, alginate and $CaCl_2$ solution)
- Excluding unstable or separable prepared gels (hydrogels containing alginate)
- The most appropriate material chosen (PVA-GK-Borax hydrogel)
- Optimization of composition ratio (GK and borax volume variations)
- Optimization of crosslinking (beaker-beaker mixing, on Petri dish spraying)
- Characterization using UV-VIS (optical properties)
- Characterization of viscoelastic properties (rheology).

4. EXPERIMENTAL PART

5.1 Chemicals

All chemicals used without further purification or modification:

- Alginic acid sodium salt was purchased from Sigma- Aldrich (Germany)
- Benzoic acid (solid) was purchased from Penta s.r.o (Czech Republic)
- Calcium chloride anhydrous (granulated) was purchased from Lach-ner (Czech Republic)
- Ethanol (96% G.R) was purchased from Lach-ner (Czech Republic)
- Gelatine (from bovine skin; type B) was purchased from Sigma- Aldrich (USA)
- Glycerol anhydrous was purchased from Lach-ner (Czech Republic)
- Natural gum Karaya was purchased from Sigma- Aldrich (USA)
- Polyvinyl alcohol (99+ % hydrolysed) was purchased from Sigma- Aldrich (United Kingdom)
- Sodium tetraborate decahydrate was purchased from Lach-ner (Czech Republic).

Chemicals with further modification:

- Sodium and potassium salt of gum Karaya, acetylated by Mrs. Nedomová according to her master's thesis [95]

5.2 Equipment

- Rheometer AR-G2 (TA instruments)
- UV-VIS spectroscopy V-730 (JASCO, Japan)

5.3 Polymer solution preparation

The substances were dissolved in fume hood during stirring and heating on a magnetic stirrer under condenser to avoid evaporation of the solution.

Solutions of high-molecular weight polymers need to be dissolved for a very long time, possibly even at an elevated temperature (60 °C), except of polyvinyl alcohol, which was dissolved for several days at 90 °C.

Borax 9% solution was prepared by dissolving 21.6 grams of borax in 150 grams of water and 90 grams of glycerol. 20 % borax solution was prepared by dissolving 20 g borax, in 37 g H_2O with 63 g of glycerol. Solution for measured samples was prepared from 11% borax solution, which were prepared by dissolving 25 g in 150 grams of water and 75 grams of glycerol.

5.4 Samples preparation

Hydrogels were prepared in different ways with different substances and optional ratios, in order to select the most suitable material.

The most common method of preparation was the beaker-beaker, used especially in case of multiple substances mixing. Prepared chemical solution were injected to two beakers, content

of beaker was stirred to homogenous solution forming. Subsequently, the crosslinking content of beaker was poured into the first beaker during intensive stirring. Another used method was used for simple gels, while crosslinking agent were injected into stirring polymer solution. A less frequent method was an application of a thin layer of substance, which was then sprayed with a crosslinking agent. Alternatively, the two layers were sprayed at the same time using a solo or double-spray (shown in Figure 16 Figure 17).

All preparations, including volume ratio, mixing method, water or substances separation are summarized in following tables 4, 5, 6 and 7, where are samples used for measure. An exception is sample 30 E6, which was measured by UV-VIS spectrometry, but after a storage for a week, the water was precipitated and gel was stiff (see Figure 15), so it was not measured by the rheometer.

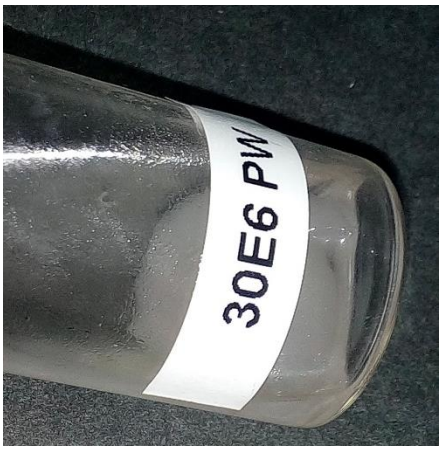

Figure 15: Sample 30 E6 over-crosslinked after week storage.

Figure 16: Double phase spray.

Figure 17: Single phase sprays.

Table 4: Overview of prepared samples.

Sample	Used substances				Volume ratio	Gel forming technique	Properties	Water separation
1	4% PVA	2% GK(K^+)	30% borax (in water)	-	10:1:1	baker-baker mixing	slightly stiff, little elastic, transaprent	no
2	4% PVA	2% GK(K^+)	30% borax	gly	20:4:4:5	baker-baker mixing	low trasnparency, stiff, non-elastic	yes
3	4% PVA	2% GK(K^+)	30% borax	EttOH	20:4:4:5	baker-baker mixing	non-transparent, white, stiff, non-elastic	yes
4	4% PVA	2% GK(K^+)	30% borax (think layer)	Gly	10:1:1:1	sprayed and mixed on borax in Petri dish	medium transparency, stiff, low-elastic	yes
5	4% PVA	2% GK(K^+)	30% borax (think layer)	Gly+ EtOH+ H_2O	10:1:1:(1:1):4	sprayed and mixed on borax in Petri dish	inhomogeneous, low-transparency, stiff, non-elastic	yes
11A	4% PVA	2% GK(K+)	9% borax	-	10:3:3	(GK+PVA) with borax in Petri dish	elastic, medium transparency, clingy, medium homogenity	no
11B	4% PVA	2% GK(K+)	9% borax	-	10:3:3	(GK+Borax) with PVA in beaker	elastic, transparency, clingy, homogenous	no
11C	4% PVA	-	20% borax	-	10:3	both substances in beaker	stiff, non-transparency, non-elastic	yes
12D	4% PVA	2% GK(K^+)	9% borax	-	5:(1:2)	PVA layer sprayed with Borax+GK solution, mixed	elastic, homogenous, transparency, clingy, 11B similar	no
12A	4% PVA	2% GK(K^+)	9% borax	-	5:(1:3)	PVA layer sprayed with Borax+GK solution, mixed	stiff, homogenous, non-transarency, overcrossed	yes
13A	4% PVA	2% GK(K^+)	9% borax	-	10:(1:3)	PVA layer sprayed with Borax+GK solution, mixed	elastic, liquid, transparent, clingy,sticky	no
14A	4% PVA	2% GK($K^{+)}$	3% Alg	0,1M $CaCl_2$	4:1:2:1	beaker-beaker mixing	medium homogenity and transparency, low-elastic	yes
14B	2% PVA	2% GK(K^+)	3% Alg	0,1M $CaCl_2$	4:0,5:2:2	beaker-beaker mixing	low homogenity and transparency, low-elastic, fluid	yes
14C	4% PVA	2% GK(K^+)	3% Alg	1M $CaCl_2$	2:1:1:1	beaker-beaker mixing	low homogenity and transparency, low-elastic, floating matter	yes, high
14D	4% PVA	2% GK(K^+)	3% Alg	1M $CaCl_2$ +9% borax	3:1:2:2:2	beaker-beaker mixing	no homogenity and transparency, non-elastic, stiff fibres	yes, high
15A	2% PVA	2% GK(K^+)	9% borax	-	2:0,4:0,5	beaker-beaker mixing	fluid, runny, sticky,	no
15B	2% PVA	2% GK(K^+)	9% borax	Gly	2:0,4:0,5:0,5	beaker-beaker mixing	low homogenity and transparency, medium-elastic, stiff	yes
16A	-	-	3% Alg	1M $CaCl_2$	1:0,5	beaker mixing	low homogenity, medium transparency, and elasticity	yes, low
16B	-	2% GK(K^+)	3% Alg	1M $CaCl_2$	0,25:0,5:0,5	(GK+Alg)+ sprayed $CaCl_2$	stiff, low homogenity, transparency and elasticity	Yes

*Gly- glycerol EtOH- ethanol Alg- alginate GK(K^+) – potassium GK salt

Table 5: Overview of prepared samples – Table 4 continued.

Sample		Substances				Volume ratio	Gel forming technique	Properties	H_2O separation
17A	2% PVA	3% Alg	2% GK(K^+)	9% borax	1M $CaCl_2$	(2:1:0.4) + (0.5:0.5)	beaker - beaker mixing	stiff, no transparency, low homgenity and elasticity	yes
17B	2% PVA	3% Alg	2% GK(K^+)	9% borax	1M $CaCl_2$	(2:1) + (0.5:0.25:0.25)	beaker-beaker mixing	stiff, no transparency, low homgenity and elasticity	yes
18A	2% PVA	-	2% GK(K^+)	9% borax	0,1M $CaCl_2$	(2:0.5) + (0.5:0.5)	beaker-beaker mixing	stiff, no elasticity, no transparency, low homgenity	yes
19A	2% PVA	3% Alg	2% GK(K^+)	9% borax	0,1M $CaCl_2$	(2:0.5) + (1:0.5:0.,25)	beaker-beaker mixing	medium-homogenity and trasnparency, low elastic, medium-stiff	yes
19B	2% PVA	3% Alg	2% GK(K^+)	9% borax	0,1M $CaCl_2$	(2:0,5) + (1:0,25:0,25)	beaker-beaker mixing	medium homogenity and elastic, trasnparency, clingy	yes, low
19C	2% PVA	3% Alg	2% GK(K^+)	9% borax	0,1M $CaCl_2$	(2:0.5) + (1:0.25:0.5)	beaker-beaker mixing	medium homogenity, low elastic, no transparency	yes, high
19D	2% PVA	3% Alg	2% GK(K^+)	9% borax	0,1M $CaCl_2$	(2:0.25) + (1:0.7:0.25)	beaker-beaker mixing	medium homogenity and elastic, medium transparency	yes,high
20A	2% PVA	3% Alg	2% GK(K^+)	9% borax	0,1M $CaCl_2$	(4:1) + (2:0.,5:0.5)	two bottle spray in petridish	no homogenity, fluid, medium transparency	yes, low
21A	2% PVA	3% Alg	2% GK(K^+)	9% borax	0,1M $CaCl_2$	(4:1) + (1:1:1)	two bottle spray in petridish	no homogenity, low transparency, stiff	yes, high
22A	2% PVA	-	2% GK(K^+)	9% borax	-	5:(1.5:2)	beaker-beaker mixing	fluid, medium homogenity and trasnaprency	no
									GK separation
22B	4% PVA	-	2% GK(K^+)	11% borax	-	(8:1:3)	beaker mixing	medium transparency and elasticity and homogenity, clingy	low, water high
30A	4% PVA	-	2% GK(K^+)	11% borax	-	(4:1:3)	beaker mixing	sticky, medium transparency, elastic, clingy, no homogenous	yes
30B	4% PVA	-	2% GK(K^+)	11% borax	-	(4:2:2)	beaker mixing	very sticky, medium transparency, elastic, clingy, no homogenous	yes
30C	4% PVA	3% Alg	2% GK(K^+)	11% borax	0,1M $CaCl_2$	(4:0.5) + (1:2:0.5)	beaker-beaker mixing	stiff, no transparency, low homgenity and elasticity,	water high
31A	4% PVA	-	2% GK(K^+)	11% borax	0,1M $CaCl_2$	4 + (1:2:1)	beaker-beaker mixing	stiff, no transparency, low homgenity and elasticity,	water high

Table 6: Overview of prepared samples – table 5 continued for all beaker-beaker mixed samples.

Sample		Substances		Volume ratio	Properties	GK separation
31B	4% PVA	11% borax	2% GK(K+)	4:(1:1)	elastic, transparency, homogenous, clingy	no
31C	4% PVA	11% borax	2% GK(K+)	6:1.5:2.5	medium stiff, low transparency, elastic	yes
31C2	4% PVA	11% borax	2% GK(K+)	6:1.5:3	sticky, medium transparency, clingy,elastic	yes, low
31C3	4% PVA	11% borax	2% GK(K+)	6:1.5:2	medium homogenity, elastic, sticky, low transparency	yes, low
31C4	4% PVA	11% borax	2% GK(K+)	6:1.5:1.5	low transparency and homogenity, sticky, elastic,fluid	yes
31C5	4% PVA	11% borax	2% GK(K+)	6:1.5:1	elastic,fluid, medium homogenity, sticky	yes,low
31B2	4% PVA	11% borax	2% GK(K+)	4:1:1.5	elastic,fluid, medium homogenity, sticky	yes
31B4	4% PVA	11% borax	2% GK(K+)	4:1:1 (slow mixing)	elastic,fluid, medium homogenity, sticky	very low
31D1	4% PVA	11% borax	-	4:2	medium stiff and transparency and elasticity	yes -water
31D2	4% PVA	11% borax	2% GK(K+)	4:2:1	medium stiff and transparency and elasticity, fluid	yes + water
30E	4% PVA	11% borax	2% GK(K+)	4:2:1	stiff, non transparent and elasticity,	yes -water
30E1	4% PVA	11% borax	2% GK(K+)	4:2.5:1	very stiff, non transparent and elasticity,	yes -water

Table 7: Overview of samples used for instrumental measurement

All samples in Table 7 are elastic, transparent, homogenous, clingy, without separation and all were measured

Samle name	Substances			Volume ratio
31C6	4% PVA	11% borax	2% GK(K^+)	6:1.5:0,5
31C7	4% PVA	11% borax	-	6:1.5
31C8	4% PVA	11% borax	2% GK(K^+)	6:1.5:0.1
31C9	4% PVA	11% borax	2% GK(K^+)	6:1.5:0.2
31C10	4% PVA	11% borax	2% GK(K^+)	6:1.5:0.3
31C11	4% PVA	11% borax	2% GK(K^+)	6:1.5:0.4
31B	4% PVA	11% borax	2% GK(K^+)	4:1:1 (slower mixing)
31B3	4% PVA	11% borax	2% GK(K^+)	4:1:1
31B5	4% PVA	11% borax	2% GK(K^+)	4:1:0.5
31B6	4% PVA	11% borax	-	4:1
31B7	4% PVA	11% borax	2% GK(K^+)	4:1:0.75
31B8	4% PVA	11% borax	2% GK(K^+)	4:1:0.25
31B9	4% PVA	11% borax	2% GK(K^+)	4:1:1.25
30E2	4% PVA	11% borax	2% GK(K^+)	4:1.5:1
30E3	4% PVA	11% borax	2% GK(K^+)	4:1:1
30E4	4% PVA	11% borax	2% GK(K^+)	4:1.25:1
30E5	4% PVA	11% borax	2% GK(K^+)	4:0.75:1
30E6	4% PVA	11% borax	2% GK(K^+)	4:1.75:1
30E7	4% PVA	11% borax	2% GK(K^+)	4:0.5:1

5.5 Sample storage

Since no stabilizers were added to the hydrogels, samples of the hydrogels were placed in the vials soon after mixing and stored in the refrigerator.

5.6 Methods of characterization

5.6.1 *UV-VIS characteristic*

Optical properties of the prepared hydrogels were analysed, while UV-VIS spectroscopy V-730 (JASCO, Japan) was used. The colour scale and absorbance of samples were measured at a wavelength from 350 to 800 nm in silica glass vials using VWCD-960 Colour diagnosis accessories with possibility of colour calculation, colour comparison, colour matching and colour difference in various colour system.

5.6.2 *Rheological analyses*

Dynamic Rheological Analyses

Cone-plate geometry with diameter of 40 mm and 2° angle was used for temperature dependence measurement of PVA-GK-BORAX hydrogels. 600 µl of hydrogel was transferred to the Peltier by syringe injection. After that, working position was set with a geometry gap of 60 µm. It was important to use solvent trap filled with water before each measurement as a prevention of evaporation of the sample solvent. The experiments were carried out under constant frequency of 1 Hz, 1% strain and the temperature was set on 37 °C. The temperature ramp for temperature sweep measurement was set from 15 to 50 °C and the rate of 0.5 °C per minute.

Steady State Rheological Analyses

For steady state rheological analyses cone plate geometry with diameter of 40 mm and 2° angle was used for measurement. Hydrogel samples tempered to laboratory temperature was transferred to the Peltier by syringe. Rheometer was prepared to the working position with geometry gap 60 µm. It was important to use solvent trap filled with water before each measurement as prevention for evaporation of the sample solvent. The experiments were carried out under the following conditions: $1s^{-1} \xrightarrow{20s} 100s^{-1} \xrightarrow{20s} 1s^{-1}$ and temperature was set at 37 °C.

5. RESULT AND DISCUSSION

5.1 Solubility of original substances

Before hydrogel preparation and characterization, it was necessary to tested solubility of original substances for its preparation in several solution. Result are summarized in Table 8.

Table 8: Overview of substances solubility.

Substances	Solvent	Degree of solubility (1-5)	Stirring	Heat at skin temperature (37°C)	Solubility time	Homogenity
natural GK	water	1	suspension	suspension, lower viscosity	long	no
natural GK, gelatine	glycerol	1	can not be mixed, dense	denser mash	very long	no
natural GK, gelatine	water , glycerol	2	can not be mixed, dense	denser mash	very long	no
Alginate	water	4	more soluble	completly soluble	short	yes
natural GK	glycerol	1	suspension	suspension	very long	no
a) natural GK	water, glycerol	2	suspension	gel suspension	very long	no
b) natural GK + glycerol	water	3	suspension	clearer gel suspension	long	yes
actelyed GK(Na^+)	glycerol, water	4	viscous liquid	clearer viscous liquid	short	yes
actelyed GK(K^+)	glycerol, water	5	viscous liquid	clearer viscous liquid	short	yes
benzoic acid	water, glycerol	1	crystals in solution	heat over 80 °C = no effect	very long	no
borax	water, glycerol	5	suspension	heat over 80 °C = clear solution	quickly	yes

During dissolution testing it was found out, that natural GK is not soluble in water, glycerol or ethanol at 37 °C. Therefore, it is necessary to used deacetylated material in each preparation. On the other hand, it was determined that borax is water soluble, but prepared solution is not stable and borax crystallizes at laboratory temperature. This problem was solved by using a stabilizer – glycerol, which also worked as a thickener and humectant.

The solubility of the benzoic acid was also tested, due to possibility to facilitate the applied gel removal by applying a thin layer of benzoic acid under sprayed hydrogel. However, this substance is poorly soluble in water and its solution remains unstable even after the addition of glycerol as in borax solution. Therefore, the acid has not been used for further work.

5.2 Hydrogel crosslinking

Because of the unknown number of functional groups of Karaya chains, the ratio of volumes of starting compounds was systematically tested. Soon after mixing, it is possible to see if the crosslinking has taken place and the gel is solid or when the gel is too fluid and flowing. Selected samples for measurement contained only PVA, borax and GK, because the water was separated when alginate and $CaCl_2$ were used. Alginate is probably not as suitable for the use of in situ crosslinked gels as GK, because it has formed gels which were more

stable in solution. In addition, hydrogels with alginate were more fragile and less homogeneous than samples without it.
For the rapid evaporation of water after application on the skin, ethanol was added to the solutions of the original materials. Unfortunately, ethanol has shown to reduce the homogeneity of the hydrogel by precipitating PVA from the solution and separating the water.

5.3 Optical properties of gels

5.3.1 *Colour scale and transparency*

The transparency and colour scale of measured samples are depicted Figure 18-Figure 26. The colour scale is determined by the parameters *a**, *b** and *L**, where *L** can be considered as a transparency rate in %. The samples were divided into three categories, which differed in the content of single-components, thus altering the overall structure of the gel. In B series, the amount of GK was changed, as well as in series C, which differs by the total volume of hydrogel but the ratio of borax and PVA is same as in the B series. In contrast, hydrogels from the E series have a constant ratio of GK and PVA, but they differ in the amount of borax.

Discussion for 31 B series

Solution of borax is totally clear without any colour and solution of PVA is transparent but has little white coloration, especially after storage in cold place. Even though GK solution is light brown coloured, near the white colour in CIE lab colour space are sample 31 B5, 31 B9 and 31 B8, which are not sample with a lowest GK contain (as shown in Figure 18). Conversely, sample 31 B6 without any GK are coloured in to light beige. On the other hand, sample 31 B8 contain the less amount of GK and second highest of PVA and borax, is situated in light purple area. Accordingly, gum Karaya possibly not influence the colour of prepared hydrogel samples. Almost all samples are near the total (zero) area, regardless the amount of several substances.

The second highest amount of GK have samples 31 B and 31 B3, while their composition is the same, but sample 31B was slower mixed. Colour of this sample is more transferred to beige area, but not much far from 31 B3 sample. In addition to, lightness *L* of both sample is almost the same, so supposable rapidity of mixing have no influence on optical properties of hydrogel.

Due to the table values and graph in FFigure 19 and Figure 20 is evident, that with decreasing amount of GK the lightness also decreases (despite of two swerved sample). Supposable, presence of GK in prepared hydrogels do not reduce their transparency, even though GK solution has little beige coloration.

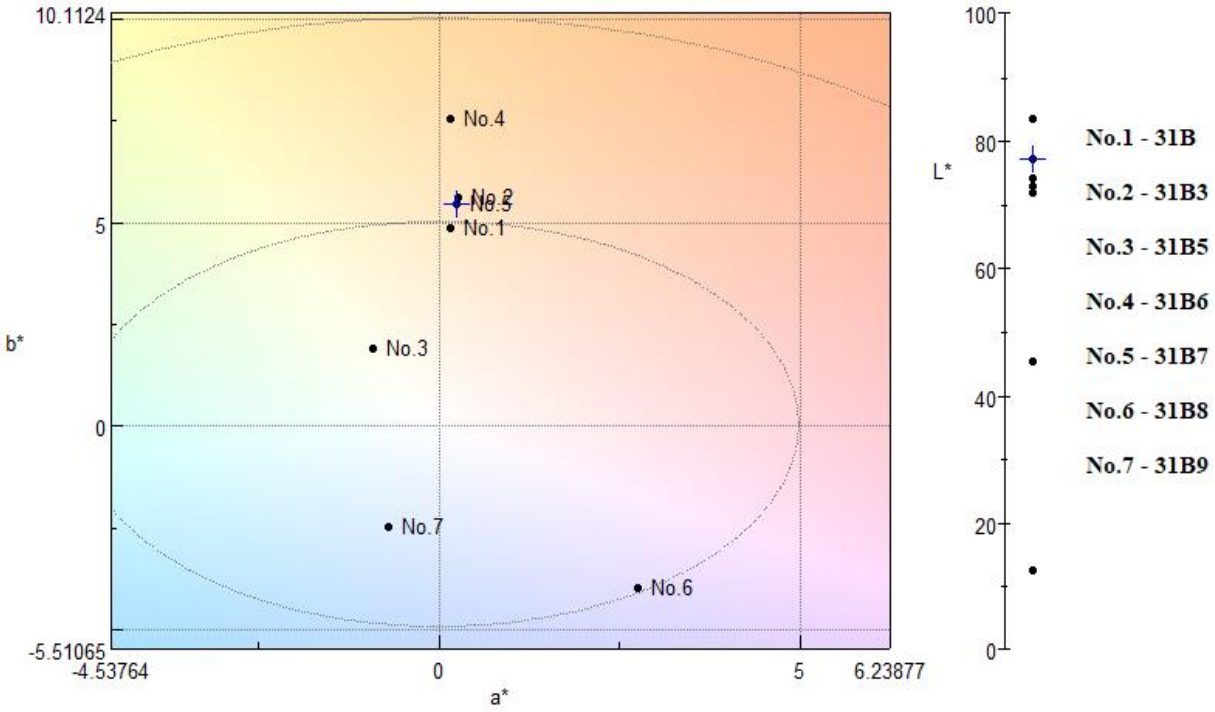

Figure 18: Colour of all B series samples.

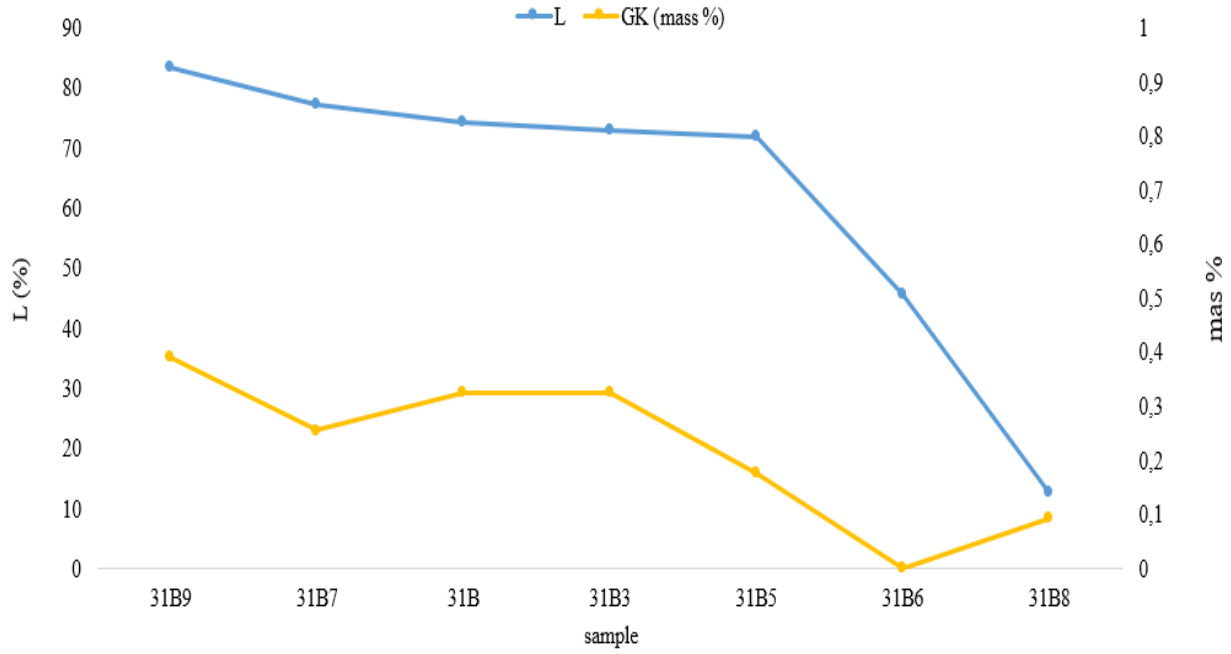

Figure 19: Graph of lightness dependence on GK mass % for B series.

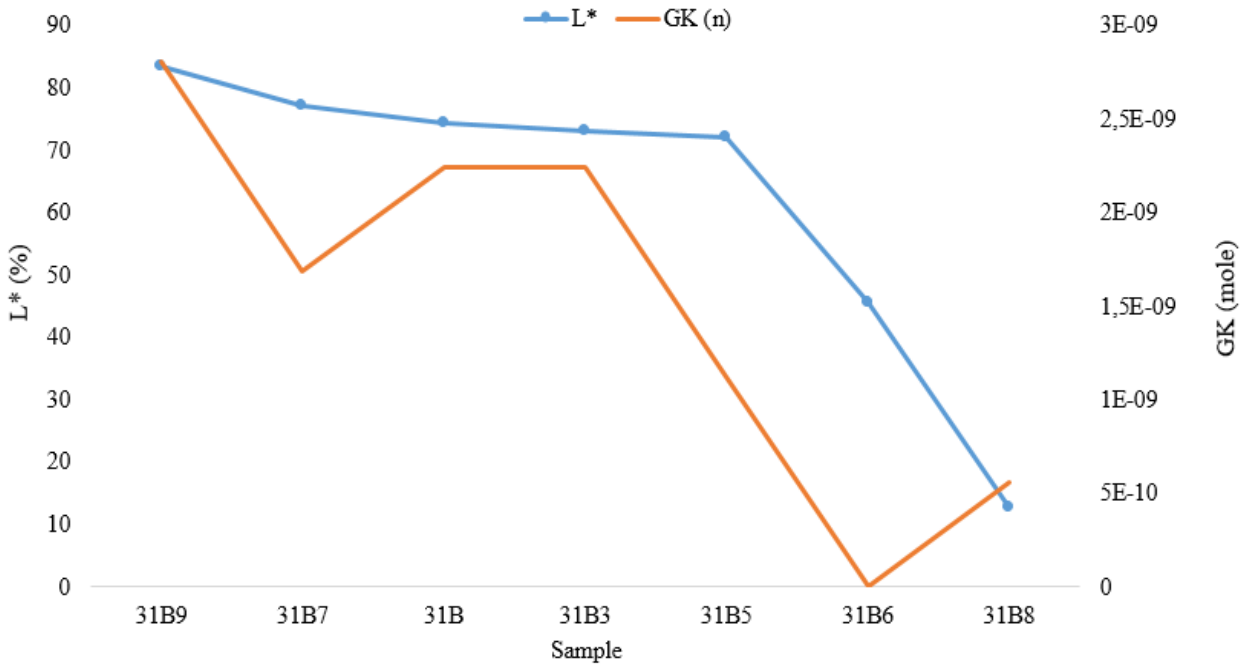

Figure 20: Graph of lightness dependence on amount of GK moles for B series.

Discussion for 31 C sample series

From graph in Figure 21 and table of substances amount is evident, that GK in hydrogels again do not influence transparency negatively. The lowest transparency (the lowest *L*) is for sample 31 C7, which do not contain any GK. Equally hydrogel with the highest amount of GK is the most transparent from C series, due to highest value of *L*. According to borax and PVA mass percent is not possible to deduce any rightfulness between L and borax and PVA contain. Again (as in the B series), sample C7 without any GK is most far away from centre of coordinate system (zero), which indicates that light beige colour of GK solution do not influence overall coloration of hydrogel. This is confirmed by other samples (C9, C11, C8), whose coordinates are located near zero.

In Figure 22: Graph of lightness dependence on GK mass % for C series is observed the *L* decreasing due to the decreasing amount of GK, except the sample 31 C8, which is unresponsive to this trend possibly due to second highest content of PVA and borax. The dependence of L on the number of moles (demonstrated in Figure 23) has a similar pattern as in the previous graph in Figure 22, while mass and mole curve have almost the same shape.

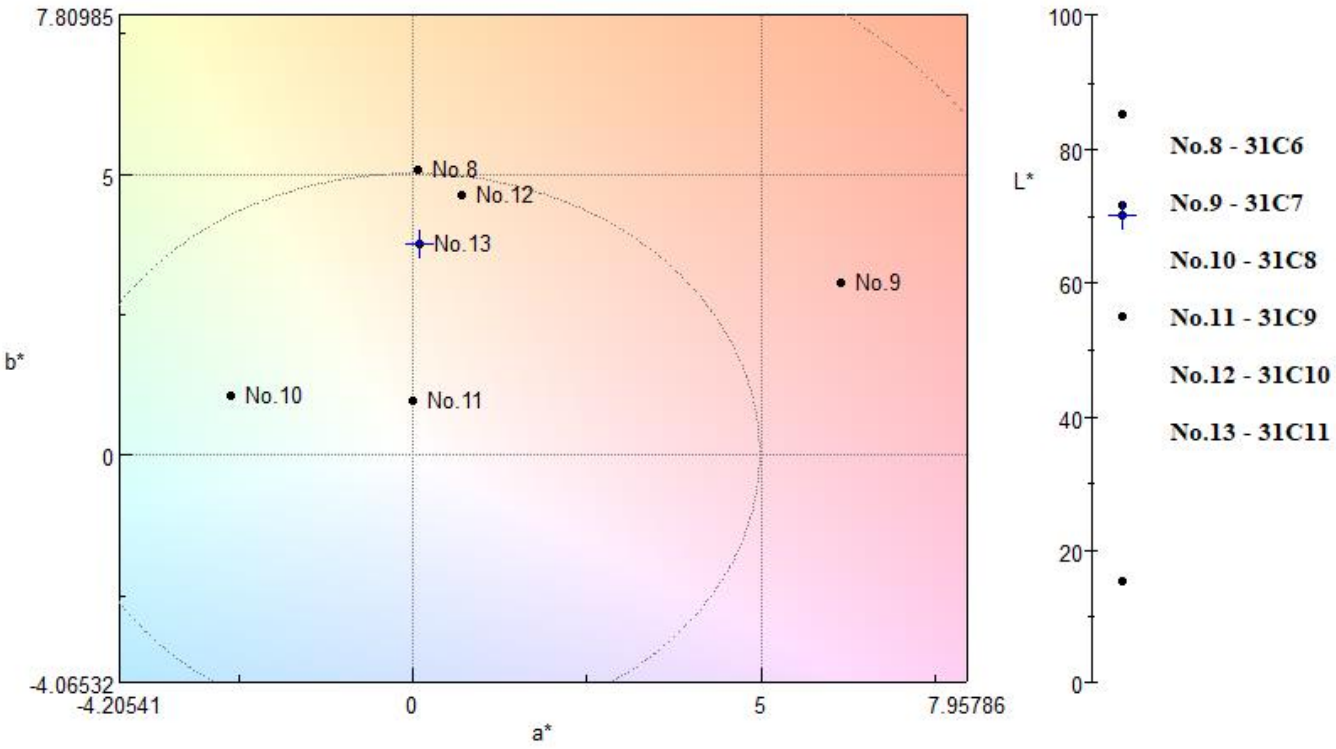

Figure 21: Colour of all C series samples.

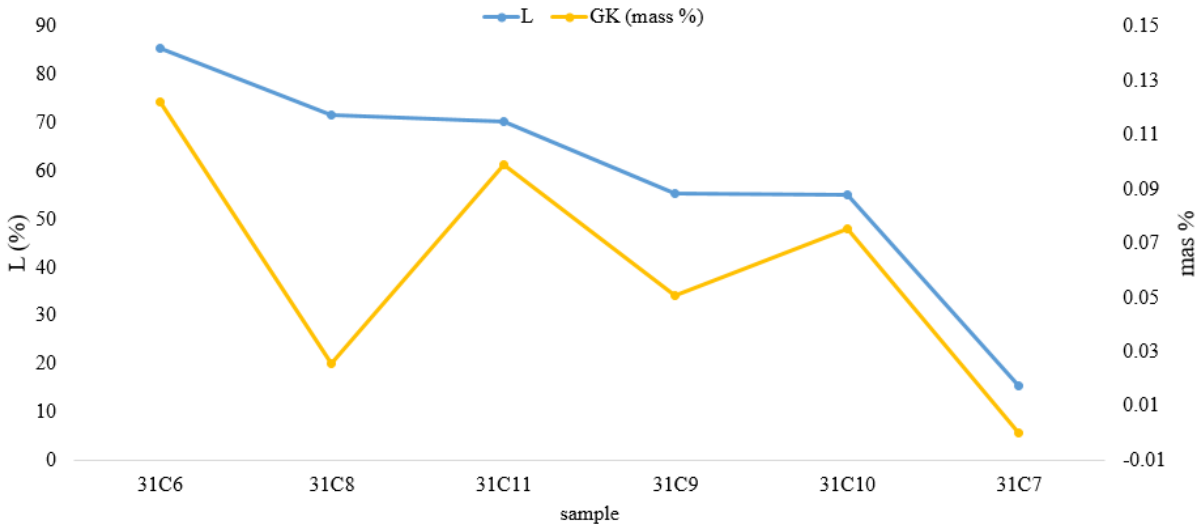

Figure 22: Graph of lightness dependence on GK mass % for C series.

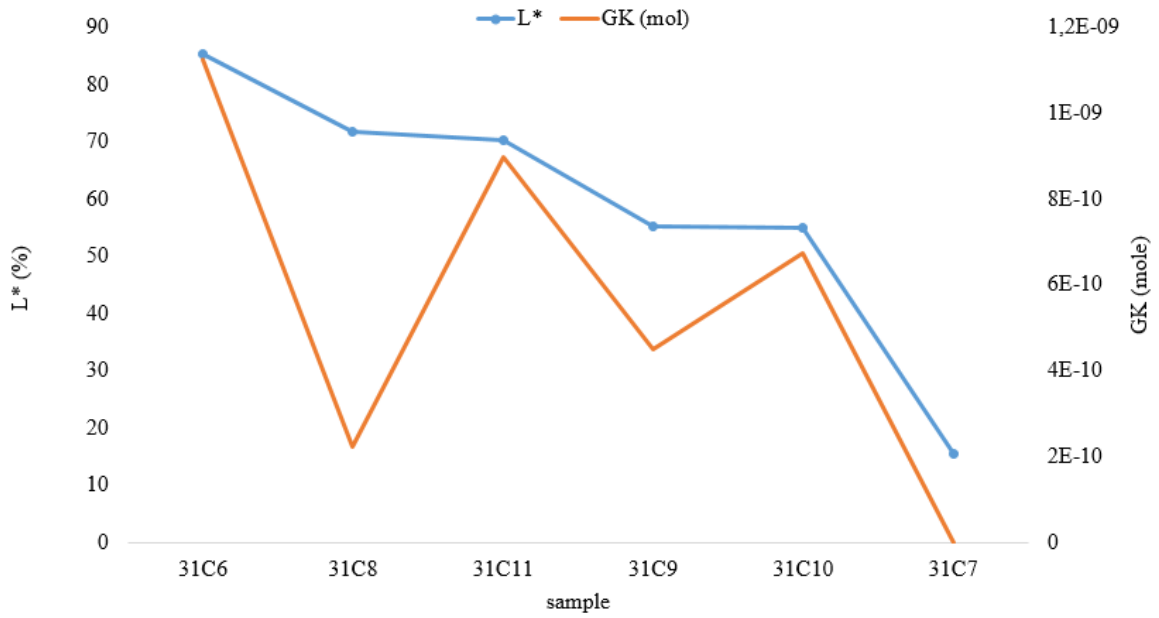

Figure 23: Graph of lightness dependence on amount of GK moles for C series.

Discussion for 30 E sample series

Almost no colouration is demonstrated by the sample 30 E4, which is situated almost in a centre of coordinates, but this sample has very low value of transparency. This is apparently caused by a small amount of GK. The most uncoloured sample (near the zero in graph) 30 E3 has the lowest transparency. It can be deduced that the light colour of the gel has no influence on the hydrogel transparency.

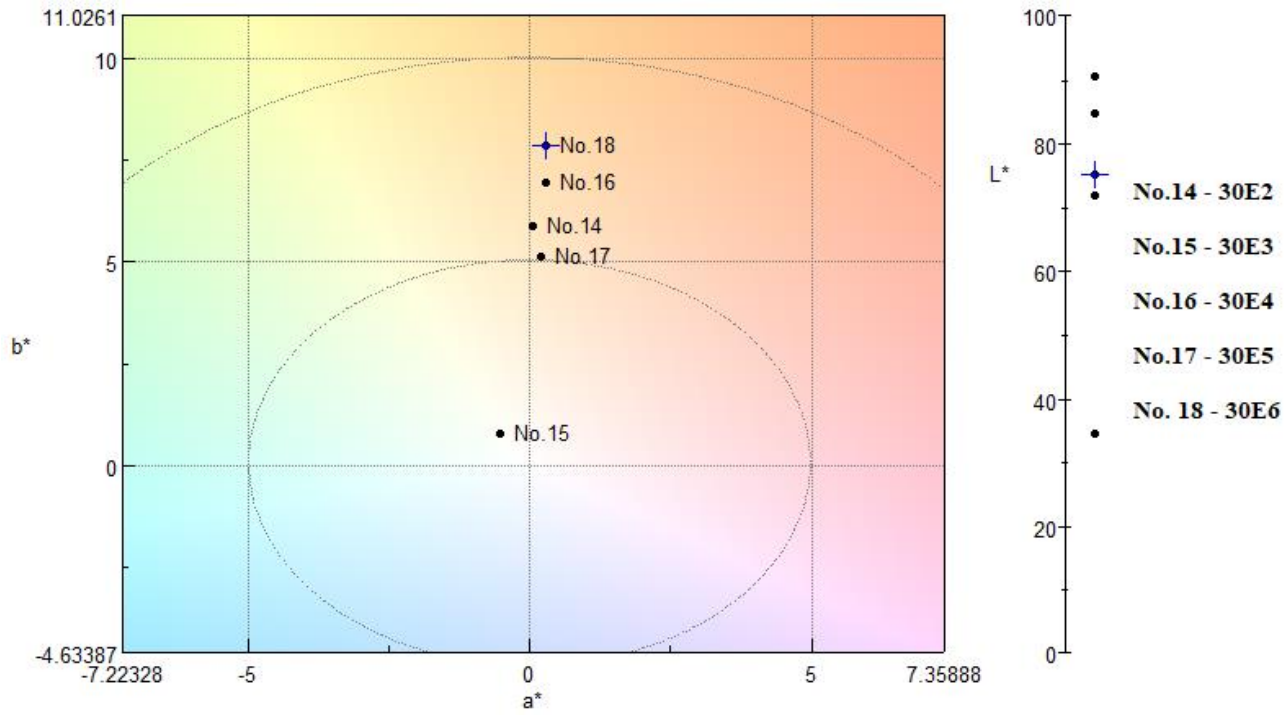

Figure 24: Colour of all E series samples.

The graphical dependence of *L* on the borax content in the samples (Figure 25 Figure 26) do not show any regular trend as same as influence of gum Karaya content in C and B series. The *L* values from these E samples were the highest among all measured samples, so that in the next investigation of PVA-Borax-GK gel, it will be possible to use a given GK and PVA ratio and to select a suitable amount of borax, for example 30 E2.

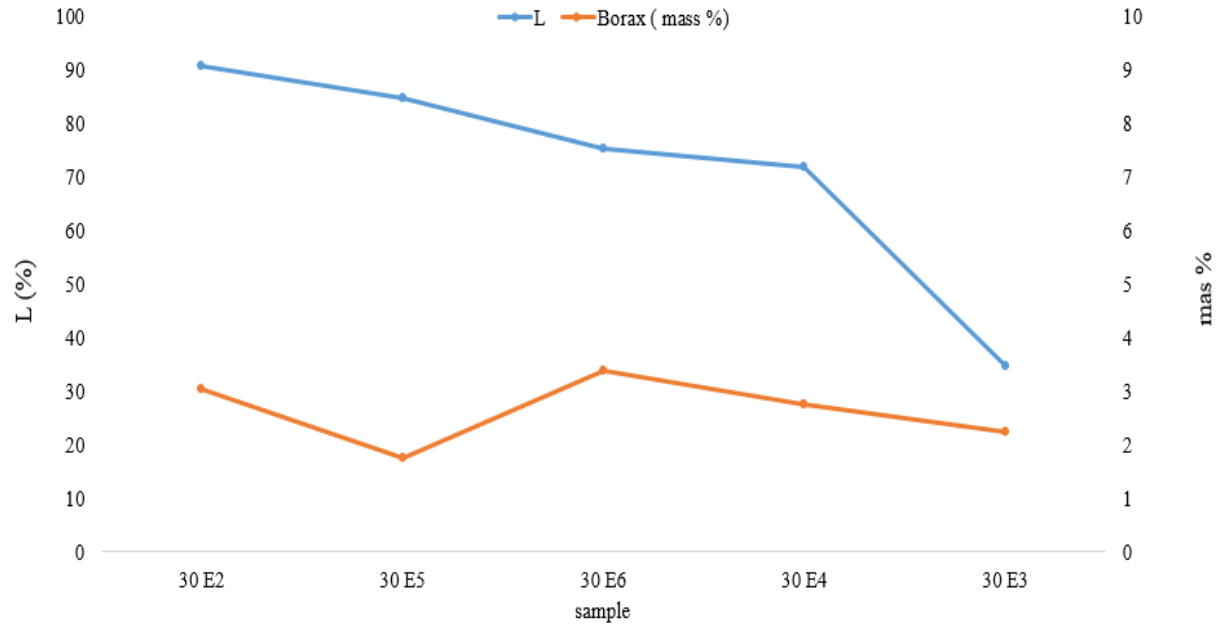

Figure 25: Graph of lightness dependence on borax mass % for E series.

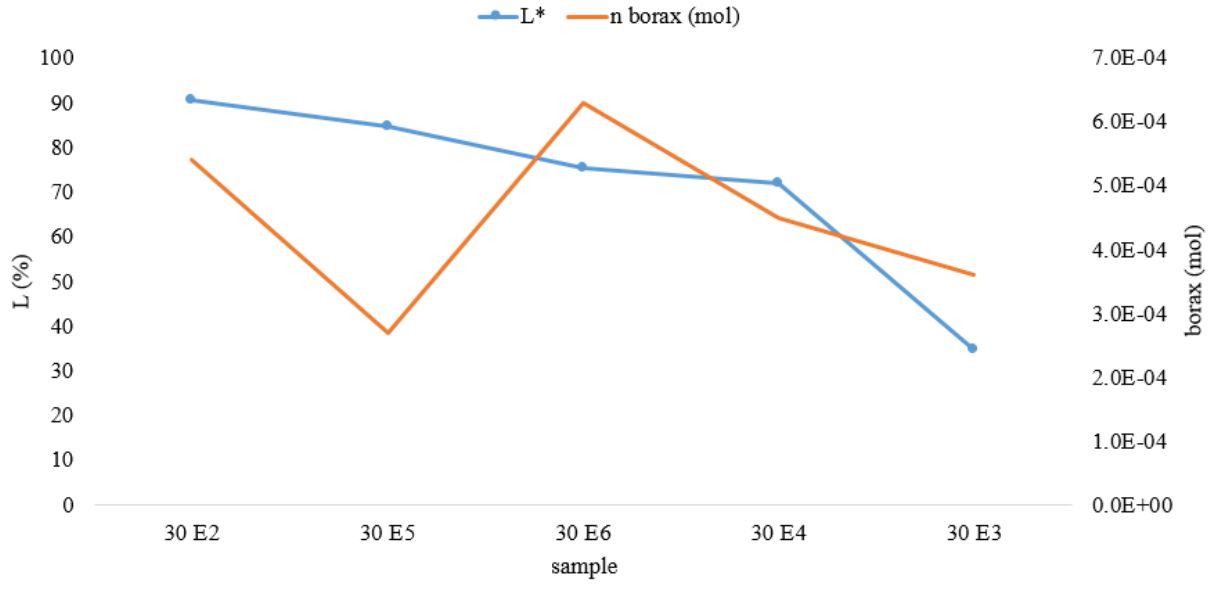

Figure 26: Graph of lightness dependence on amount of borax moles for E series.

Measured data show no negative influence of GK content and gel transparency, conversely more GK improved the gel transparency. As for the colour, it is seen macroscopically (by the human eye) that the brown GK solution slightly discolours the prepared gel where the gel colour is slightly yellowish-brown with higher GK content. Transparency will depend on the

optimal ratio of the substances and crosslinking density. The reproducibility of the measurements is low, because small manipulation with the prepared hydrogel formed bubbles and pores stable in the vial for a longer time.

5.3.2 *Absorbance*

In B series the amount of GK was changed, while the ratio of PVA and borax was equal to 4:1. The highest content of GK has sample 31 B9, the lowest 31 B8 and sample 31 B6 does not contain any gum Karaya. The amount of gum Karaya in the samples further decreases in order B9 > B = B3 > B7 > B5 > B8 >B6 and absorbance decreases in order: B6 > B5 > B9 > B3 > B> B8 > B7. Samples 31 B and 31 B3 have the same contain, which is also confirmed by measurement, because their curves are almost the same (shown in Figure 27). The highest absorbance has the sample without GK 31 B6, and with low amount of GK 31 B5. This samples confirmed the theory that absorption increases with increasing content of GK, but other samples exclude it. Therefore, for the B series, there is no correlation between absorbance and sample composition, but samples with none or the lowest GK amount have the absorbance very high. The dependence between the PVA and borax contents has also not been proven, so it can be concluded that the use of the high absorbance sample which include GK will be most appropriate, such as 31 B5. But this sample has low transparency, so sample 31 B9 will be more suitable for another investigation. The absorbance can be affected by the structure which varies according to the mixing and gel formation process. All hydrogels absorb more at the region of wavelength of UV radiation than at region of visible light. Especially absorbance of samples 31 B6 and 31 B5 is very high, so these hydrogels can partly protect from the influence of UV radiation. Visible light, which accelerate the degradation of organic matters, was partly absorbed by all prepared hydrogels.

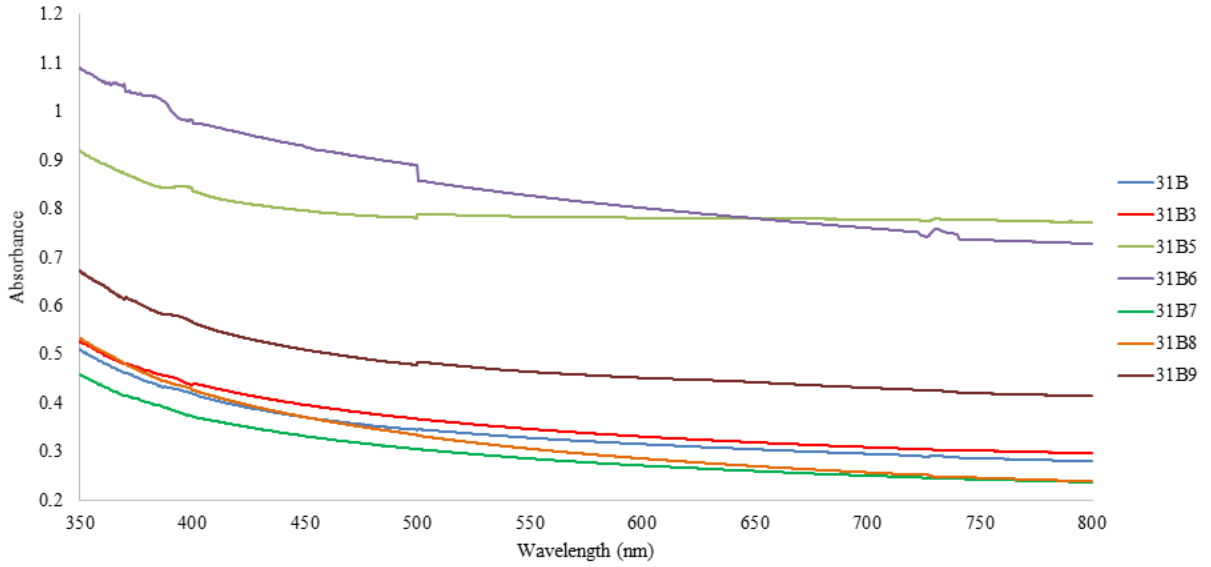

Figure 27: Graph of absorbance dependence on wavelength.

In C series the amount of GK is changed, while the ratio of PVA and borax is same as in series B, but the volume of the whole hydrogel increased, which causes GK concentration decrease. In Figure 28 is shown, that the curves of the highest and lowest amount of GK are very close and another samples also refute any dependence between absorbance and GK content as in the case of the B series. The light absorbance has similar characteristics as in the case of the B series. The highest absorbance achieves a sample with middle values of the contents of all three substances, this ratio may well be suitable for further use, however, in this C series, the better the transmissibility is, the less light the hydrogel absorbs.

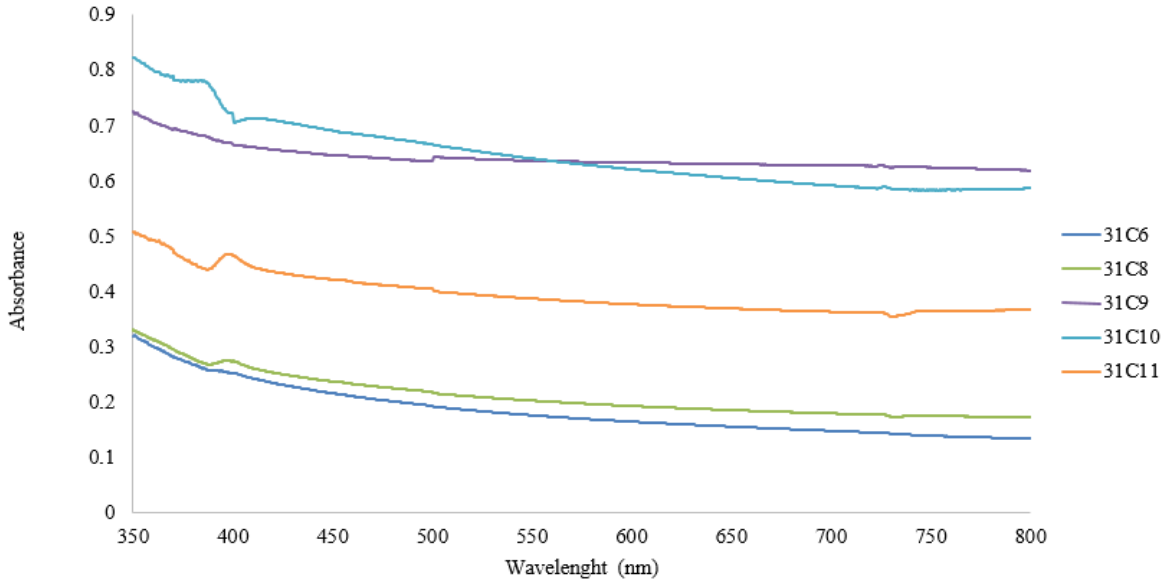

Figure 28: Graph of absorbance dependence on wavelength for C series.

In E series the amount of borax was changed, while ratio of PVA and GK was stable (4:1). The amount of borax (crosslinking agent) in the samples further decreases in order: E6> E2> E4> E3> E5 > E7. Sample 30 E7 with lowest borax concentration responses to the highest absorbance, but all other samples refute that absorbance decreases with borax increasing and conversely (see Figure 29Figure 29).

Apparently there is another factor which greatly affects the absorbance of the prepared hydrogels. Since they are substances that change handling properties, the placement of gels in the cuvettes can also affect absorbance. The structure of the prepared hydrogels could affect absorbance, however, samples of the same composition but prepared at different rates and stirring times (such as 31 B and 31 B3 have almost identical the absorbance curve). Absorbance will depend on the optimal ratio of the substances and crosslinking density and also on the homogeneity of hydrogel.

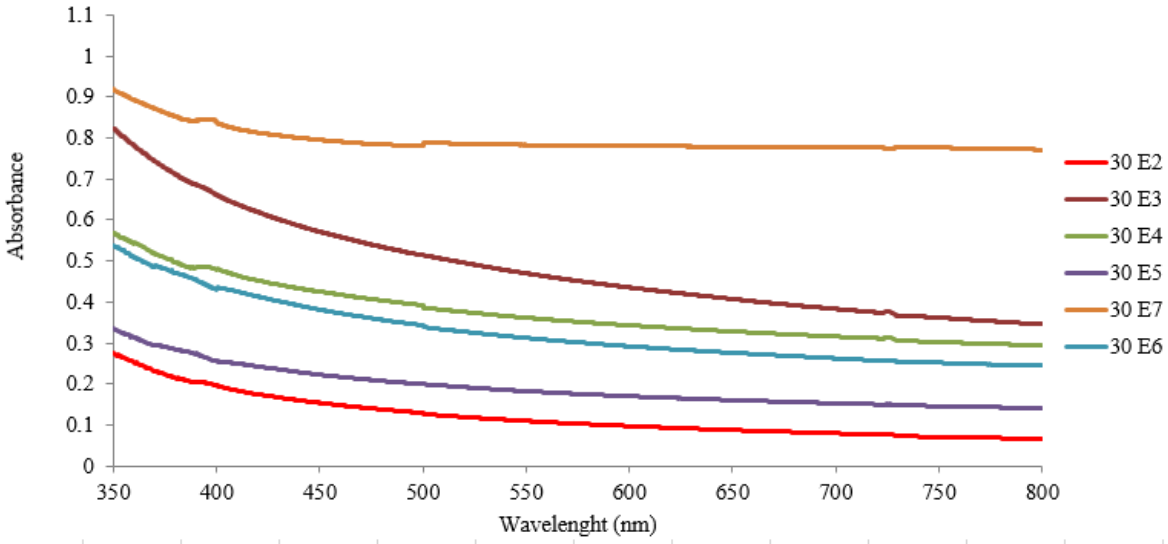

Figure 29: Graph of absorbance dependence on wavelength.

5.4 Hydrogel visco-elastic properties

5.4.1 *Thixotropy*

A representative sample 30 E3 was chosen on which it was measured whether thixotropic hydrogels were prepared or not. A hysteresis loop appears on the flow curve of PVA-Borax-GK system. The portion of the stress-increasing curve does not have the same shape and does not coincide with the stress-reducing curve, so measured hydrogel is certainly thixotropic. The shape of the curve follows that these hydrogels have thixotropic properties as shown in Figure 30. Thanks to thixotropic treatment, the prepared hydrogels can be injected as demonstrated in Figure 30. Significant thixotropic properties are commonly observed in some gels, both reversible and irreversible, with physical joints, which are the forces attaching the original dispersion particles to the mesh structure. If they are weak, the gel can be converted to sol again by shaking or repeated pressure, when mechanical effects interfere with the weak bonds between the particles. If the liquefied sol stays at rest, the bonds are slowly renewed and new gelation occurs and the viscosity gradually becomes the original value. This phenomenon was also noted for these PVA-borax-GK gels, after measuring the gel was removed from the rheometer in almost the same state.

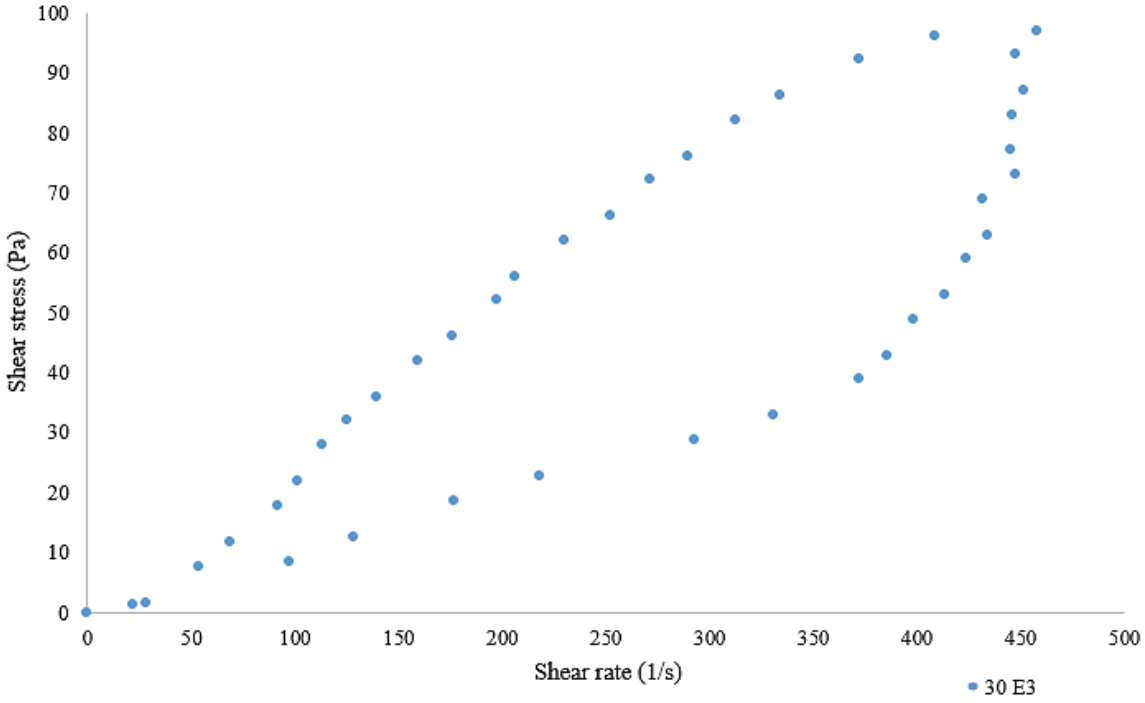

Figure 30: Graph of shear stress dependency on shear rate for thixotropy demonstration.

Figure 31: Injectability test of the prepared hydrogel.

5.4.2 *Strain sweep*

It was necessary to find a suitable strain level at which the G´´ module value would be constant, so that in the next measurement the G´´ value would depend only on another variable (frequency, temperature, etc.). A series of samples E, with a constant ratio of PVA : GK (4:1) and varying borax content, were used for measurement. For comparison, the sample 31 B6 is shown in the graph (Figure 32), which does not contain any GK.

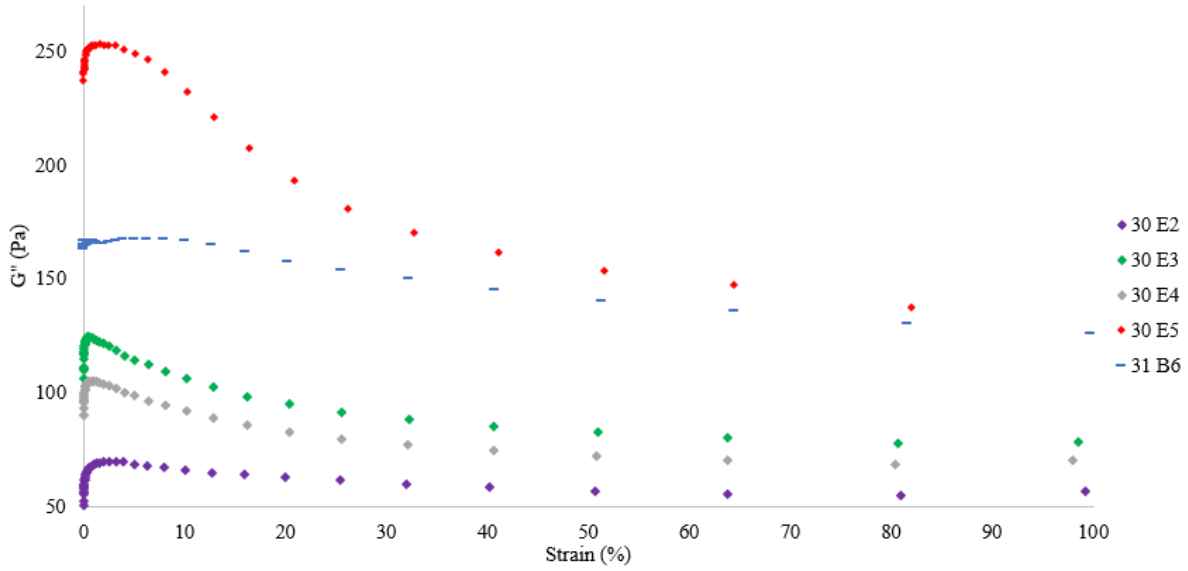

Figure 32: Graph of G" module depended on strain increasing for E series

The borax content of the samples decreases in the following order: 30 E2 >30 E4 >30 E3 >30 E4 > 30 E5. The measurement results show, that the smaller the borax concentration, the greater the value of the module is and so the firmer the hydrogel is. The amount of borax that exceeds the amount required for gelation therefore disrupts the structure of the hydrogel and the network is not so stiff, even though the hydrogel is not over-crosslinked resulting in no water separation.

Because of the modulus changes with at the different strain values, most of the linear area was selected, about 1%, as shown in

Figure 33. All other measurements were therefore measured at strain values equal to 1%

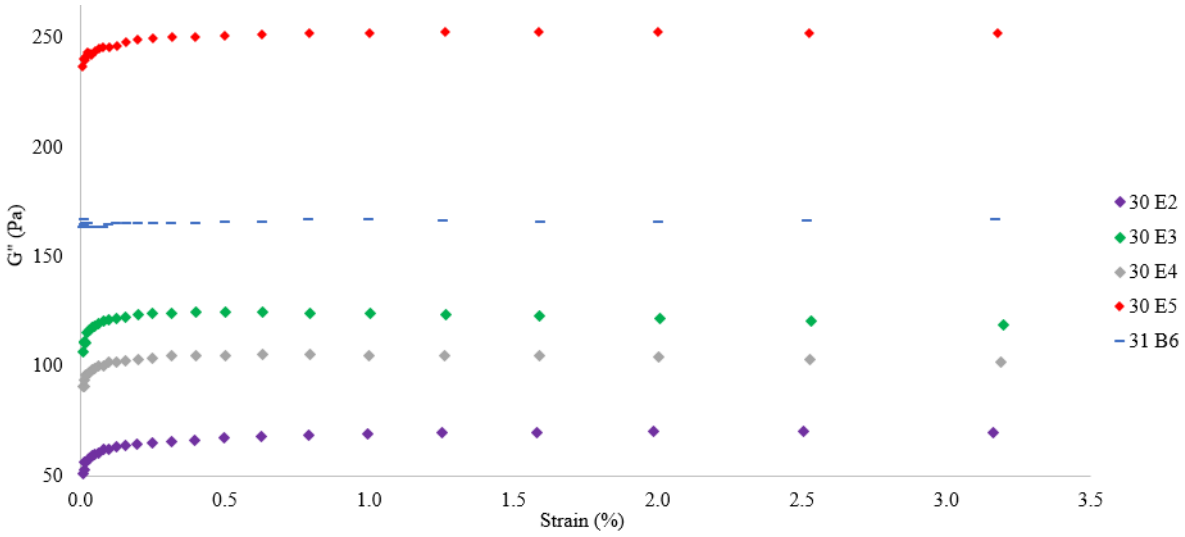

Figure 33: Graph from figure 19 focused on lower strain values

5.4.3 *Frequency sweep*

The sample E series was used to determine the linear frequency range. The linear area was unfortunately not found in any sample, even at high (150 Hz) or low frequencies. As shown in Figure 34, module G' increases or decreases throughout the range of used frequencies.

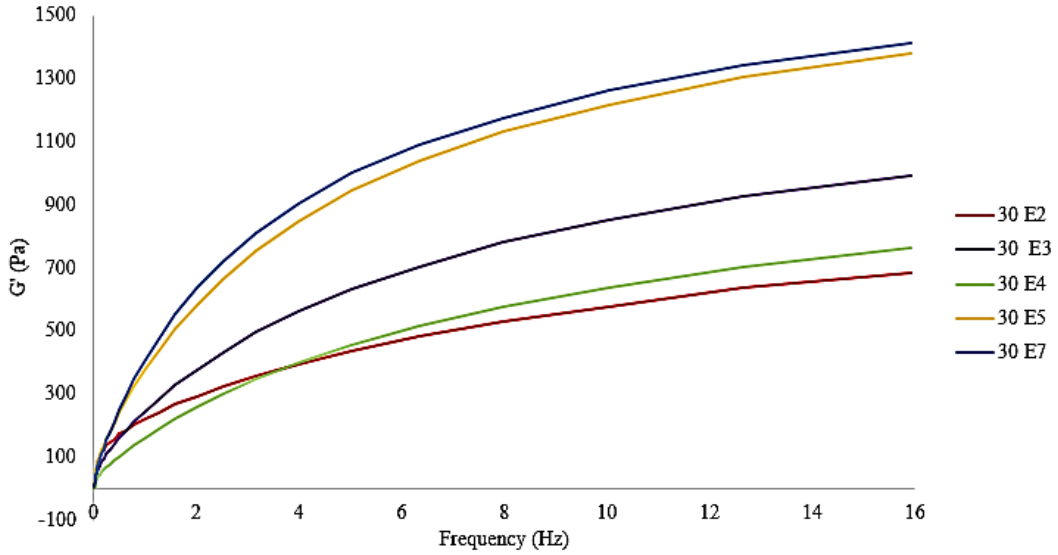

Figure 34: Graph of frequency sweep for E series (at 37 °C)

The highest G' (elastic) module was measured for sample 30 E7, and the lowest module for sample 30 E2, as shown in figure 21. With the increasing concentration of borax, the hydrogel strength decreases, as demonstrated in strain sweep section.

It was also measured frequency sweep of two samples from two different series with the same volume ratio of PVA and borax, except that sample 31 B6 contained no GK. Even for a single sample, no linear frequency domain was found again. Graph for this measurement is shown in Figure 35.

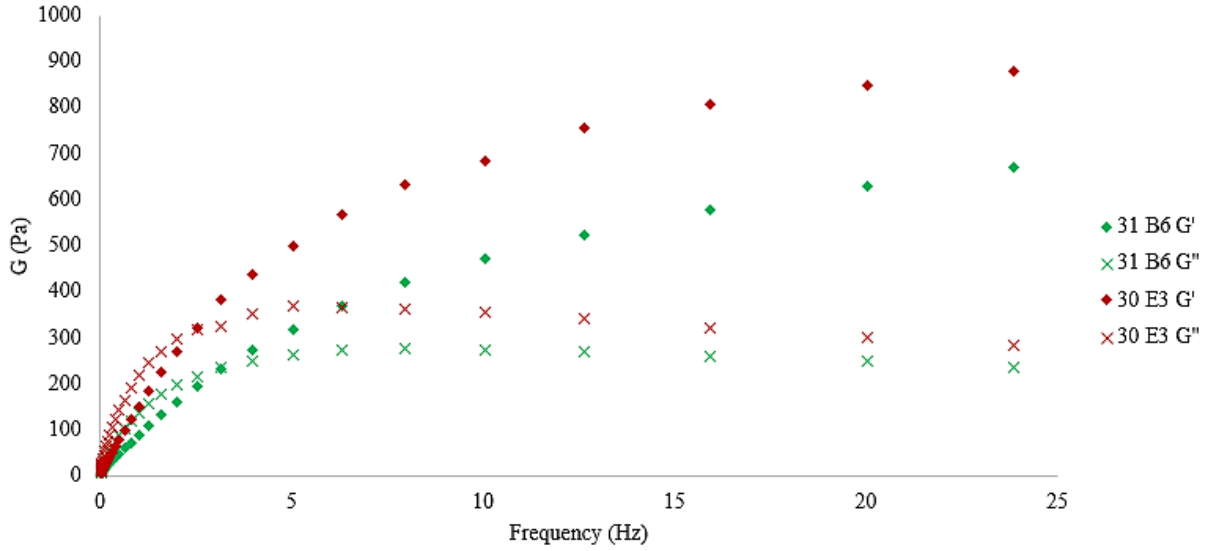

Figure 35: Graph of frequency sweep measured for 30 E3 and 31 B6

During measuring the viscoelastic module was superior to the elastic for all samples. By increasing the detail of the previous graph (figure 22) it is possible to see that at a certain frequency (2,5 and 3,2) over time, the elastic modulus G' dominated over G" .The reason is perhaps that at an increased frequency causes de-crosslinking of hydrogel, therefore changes degree and the lifetime of the crosslinking of hydrogel [38] But while the tension is removed, the gel returns to its original state because it is reversible. The samples have same ratio of borax and PVA, but 30 E3 contain also several amount of GK. The measured values of the modules show that the hydrogel containing GK is more viscoelastic, however, it withstands less pressure, because the de-crosslinking was occurred at a lower frequency than the sample B6 (see Figure 36).

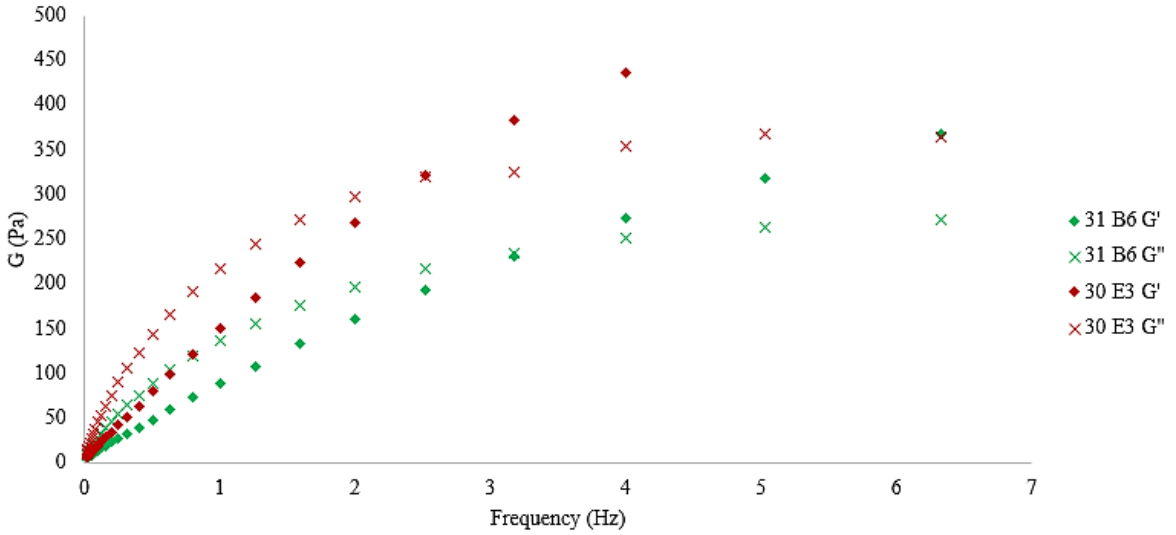

Figure 36: Detailed graph of frequency sweep measured for 30 E3 and 31 B6

In the case of prepared hydrogels, the G´ and G´´ modules always seem to rely on the used frequency, because no constant frequency region has been detected. For this reason, further measurements were performed at a frequency of 1 Hz, which was used in a scientific article containing rheological properties of hydrogels [38], which were very similar to the hydrogels in this work, therefore PVA - borax hydrogels with dispersed cellulose nanoparticles. In the study was also failed to find a constant frequency area.

5.4.4 *Temperature sweep*

A change in the viscosity behaviour of the prepared hydrogel at the rising temperature was measured, to determine the structure changes of the hydrogel after application. Hydrogel sprayed on wound change its temperature due to temperature difference of laboratory (20 °C) and skin temperature (36.8 ° C).

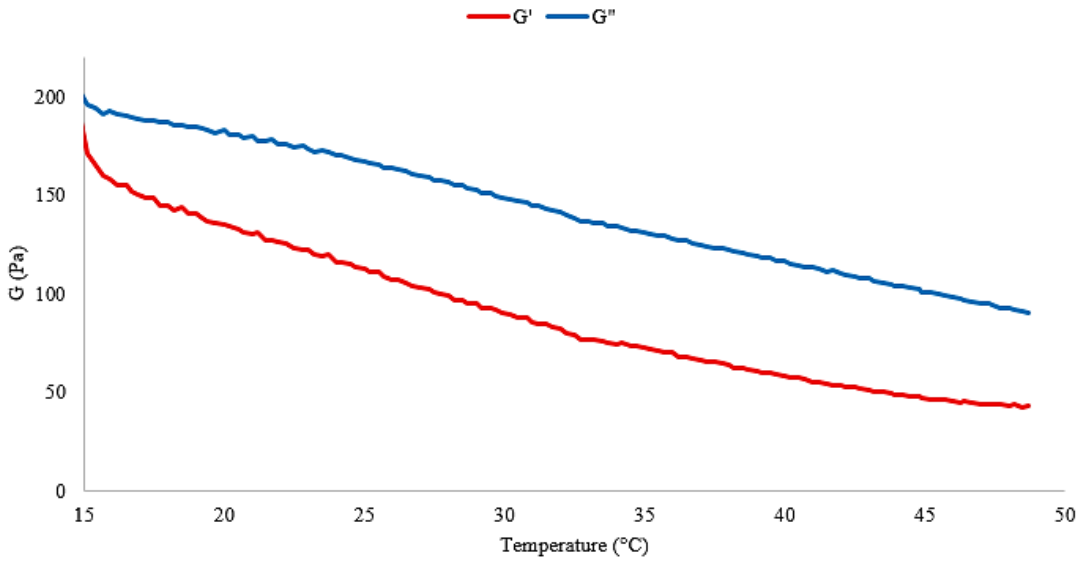

Figure 37: Graph of the G´ modulus change by the temperature increasing

The graph (Figure 37) shows, that the gel loses its elasticity with temperature increasing, apparently the gel should be well-adaptable to human skin. The disadvantage is probably the decrease of the viscoelastic modulus, because this perhaps means that after application to the skin, the gel starts to spread out and changes its volume or shape.

5.4.5 *Gelation*

Appropriate parameters were set for measuring the prepared hydrogels, i.e. the frequency was 1 Hz; strain 1% and temperature was adjusted to a physiological on 37 ° C. The strength and elasticity of the hydrogel was measured as a function of time.
The strength and elasticity of the hydrogel was measured as a function of time. A sample 30 E3 was compared with 31 B5, which has the GK content 46 mass % less than sample 30 E3.

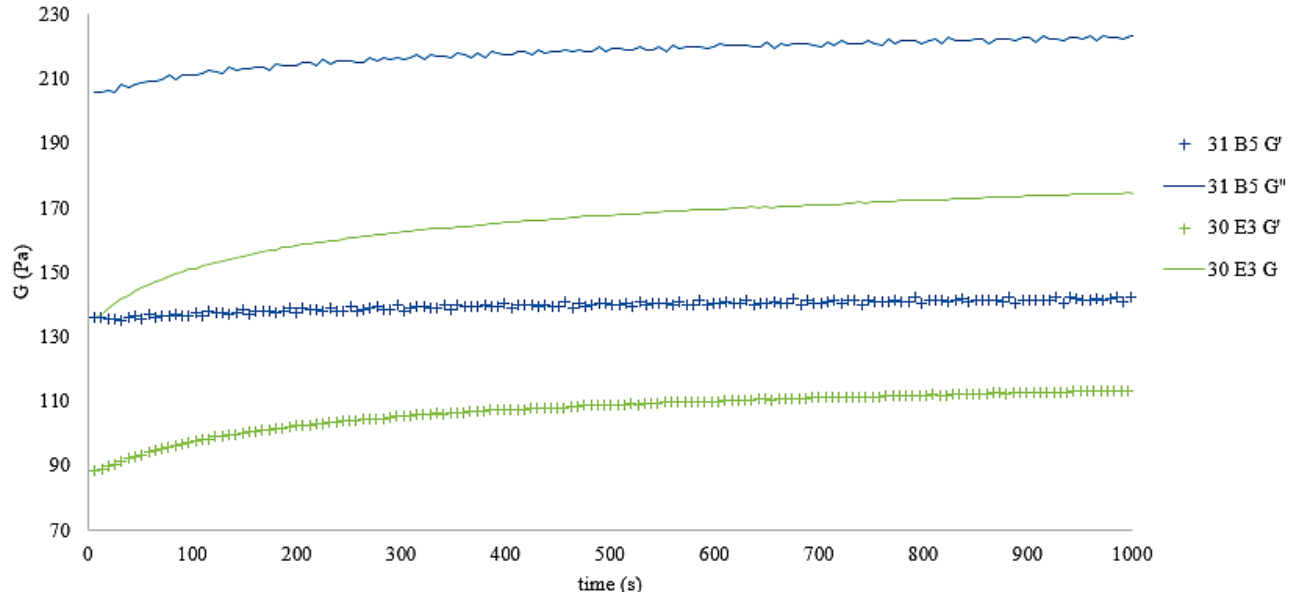

Figure 38: Graph of G modules change during the time, comparing two samples

The Figure 38 shows, that no gelation occurs anymore, because the curves of modules did not cross in any sample. Because gelation takes place within a few seconds after hydrogel mixing, this result was expected. The graph also shows that hydrogel 31 B5 has both modulus higher than 30 E3, in that is more firmer, probably due to a lower GK content that can reduce gel strength as demonstrate in Figure 36.

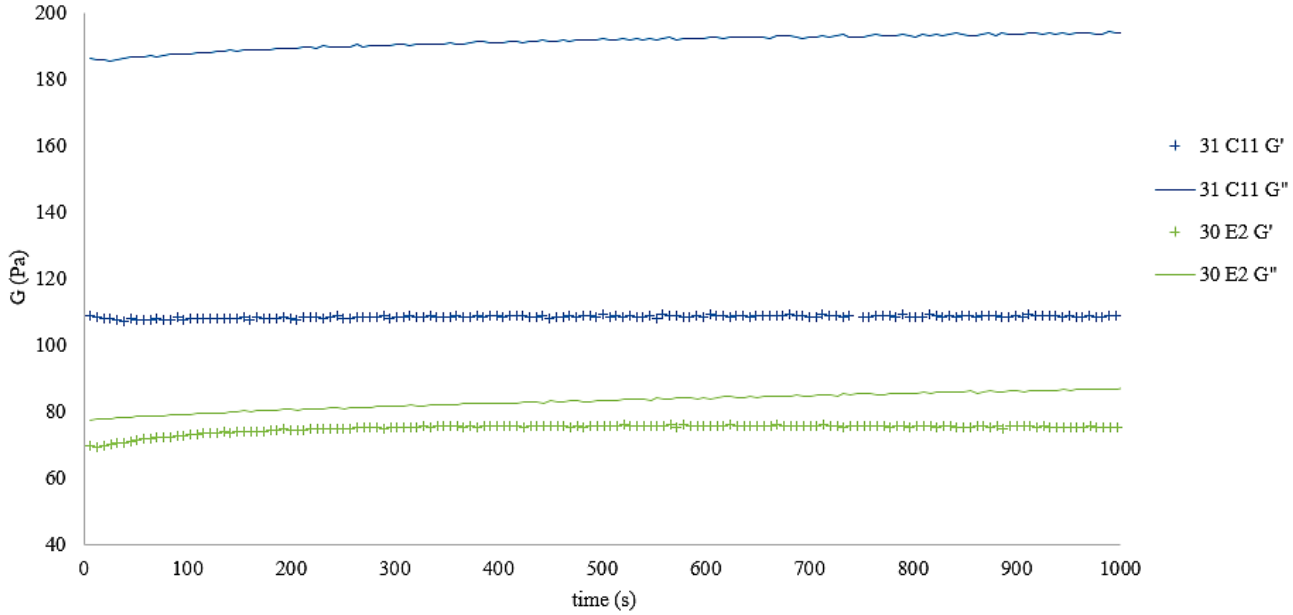

Figure 39: Graph of G modules change during the time, comparing two samples

The same measurement were made for the samples 30 E2 and 31 C11, again with different amount of GK, but in lower concentration due to larger volume of hydrogel 31 C11. As in the previous case, hydrogel with a lower GK content was more resistant than gel contains 67 mass % more than low-contain GK sample. The difference is much higher than in the previous case, as shown in Figure 39, i.e. the GK content has a significant influence on hydrogel mechanical properties.

The gelation of hydrogels with constant PVA and GK ratio was also measured, where the sample 30 E3 contain over 35 mass % than 30 E2. As can be seen from the graph (Figure 40), a hydrogel containing more borax is less firm, because it has considerably lower values of both G modules.

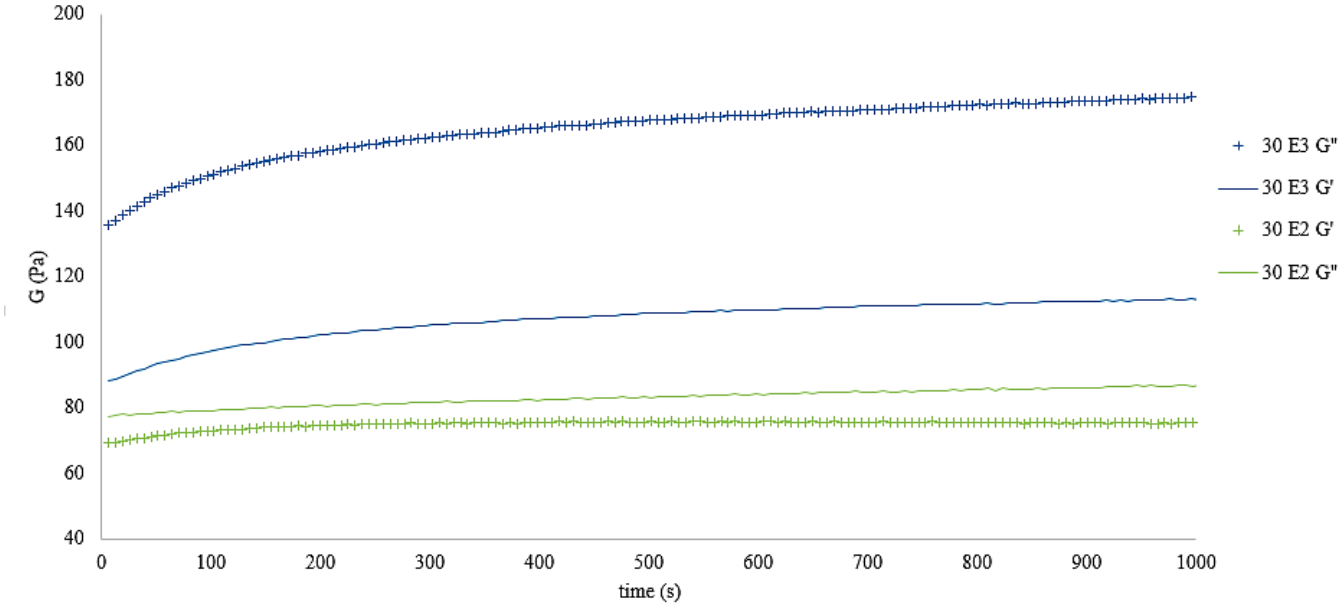

Figure 40: Graph of G modules change during the time, comparing two 30 E samples

6. CONCLUSION

Presented work was focused on the sprayable hydrogels which would facilitate application in wound healing and burns. Due to the limitation of synthetic materials and increase the usage of renewable natural substances, widely accessible polysaccharide gum Karaya was added to PVA-Borax hydrogels. The use of this cheap and affordable material could help with the commercialization of sprayable hydrogels, which has been a great deal of interest in medicine and science.

An attempt to introduce another renewable natural material into the gels was successful, however, alginate was not chosen as a suitable polysaccharide in the final measurable samples because it disrupted the homogeneity of hydrogels by a considerable water separation. Glycerol has been added as an additive which, after application, keeps the moisture in wound ambient, and acts as a stabilizer for a self-crystallising solution of borax

Hydrogels sprayings from PVA, borax and gum Karaya was carried out in petri dishes by two single-phase sprays or a single two-phase spray, but which was clogged with a viscous solution of PVA. The spraying method needs to be further optimized, so that both phases (polymeric and crosslinking) form two homogeneous, immediately gelling layers in the same proportion of substances as in the beaker-prepared samples.

Optical properties of these hydrogels measured via UV-VIS spectroscopy showed dependence of GK amount on its transparency and colour. The GK content does not reduce transparency even though the solution is not colourless as PVA and borax solution.

During rheological measurements, it was found that prepared PVA-Borax-GK hydrogels are thixotropic. It follows that the apparent viscosity of these thixotropic hydrogels decreases due to long-term shear stress. Unfortunately, no linear frequency values were found during optimization. The viscoelastic modulus for all the samples measured was higher than elastic, which is probably due to the fact that, although the hydrogels are elastic, their elasticity is not large enough to return to materials initial state after deformation. The prepared gels are highly viscous materials with tendency to "flow" and stretch while a horizontal position is changed.. The temperature sweep shows, that the gel loses its elasticity with temperature increasing, apparently the gel should be well-adaptable to human skin, but after application to the skin, the gel perhaps changes its volume or shape. During the gelation time measurement it was confirmed that GK contain can reduce the hydrogel strength. The mechanical resistance and strength of gels is also affected by the borax content. The increasing borax concentration decreases the value of the G modules, so the hydrogel is less firm, which was proven during gelation time and strain sweep measurement.

This work could be the basis for the further development and research of the prepared materials and the optimization of their measurements. These hydrogels can be sprayed, however, it is not easy to optimize the sprayed amount of both substances so as to form a homogeneous dressing. The biggest problem is the viscosity of the PVA solution, the application of which by appropriate spray is a stimulus to the further investigation. Also, based on the findings, the properties of PVA-BORAX-GK hydrogels can be further modified by adding drugs or nanoparticles for wound healing to obtain an inexpensive, sprayable healing hydrogels suitable for commercial use.

7. REFERENCES

[1] SINGH, Baljit, Lok PAL, R.C.M DE PAULA, J.P.A FEITOSA a H.C.B PAULA. Development of sterculia gum based wound dressings for use in drug delivery: note II. *European Polymer Journal*. 2008, 1968, **44**(10), 3222-3230. DOI: 10.1016/j.eurpolymj.2008.07.013. ISSN 00143057.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0014305708003418

[2] BHATTACHARYA, A. Grafting: a versatile means to modify polymersTechniques, factors and applications. *Progress in Polymer Science*. 2004, **29**(8), 767-814. DOI: 10.1016/j.progpolymsci.2004.05.002. ISSN 00796700.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S007967000400067X

[3] POUCHLÝ, Julius. *Fyzikální chemie makromolekulárních a koloidních soustav* [online]. Vyd. 3. Praha: Vydavatelství VŠCHT, 2008 [cit. 2018-05-05]. ISBN 978-80-7080-674-6.

[4] AHMED, Enas M. Hydrogel: Preparation, characterization, and applications. *Journal of Advanced Research*. Dokki - Cario University in Egypt: National Research Centre, 2013, **6**(2), 105-121. DOI: 10.1016/j.jare.2013.07.006. ISSN 20901232. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2090123213000969

[5] BILANOVIC, Dragoljub, Jeanna STAROSVETSKY, Robert H. ARMON a Yi-Hsiang LIN. Preparation of biodegradable xanthan–glycerol hydrogel, foam, film, aerogel and xerogel at room temperature: Effect of sorbitol and glycerin on the stability, swelling and mechanical properties. *Carbohydrate Polymers*. 2016, **148**(2), 243-250. DOI: 10.1016/j.carbpol.2016.04.058. ISSN 01448617.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0144861716304192

[6] DUFLOT, Anastasia V., Natalia K. KITAEVA, Vladimir R. DUFLOT, Shunji NOMURA a Wel-li WU. Radiation-chemical preparation of poly(vinyl alcohol) hydrogels. *Radiation Physics and Chemistry*. 2015, **107**(10), 1-6. DOI: 10.1016/j.radphyschem.2014.08.002. ISSN 0969806X.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0969806X14003302

[7] NEUMANN, P. M., B. ZUR a Y. EHRENREICH. Gelatin-based sprayable foam as a skin substitute. *Journal of Biomedical Materials Research*. 1981, **15**(1), 9-18. DOI: 10.1002/jbm.820150105. ISSN 0021-9304.
Available from: http://doi.wiley.com/10.1002/jbm.820150105

[8] TØNNESEN, Hanne Hjorth a Jan KARLSEN. Alginate in Drug Delivery Systems. *Drug Development and Industrial Pharmacy*. 2002, **28**(6), 621-630. DOI: 10.1081/DDC-120003853. ISSN 0363-9045.
Available from: http://www.tandfonline.com/doi/full/10.1081/DDC-120003853

[9] RIMMER, S. Synthesis of hydrogels for biomedical applications: control of structure and properties. Biomedical Hydrogels. Elsevier, 2011, 2011, 51-62. DOI: 10.1533/9780857091383.1.51. ISBN 9781845695903.
Available from: http://linkinghub.elsevier.com/retrieve/pii/B9781845695903500035

[10] C. HYMES, Alan, Lincoln T. ONG a Garry R. PERSONS. Drug dispensing device for transdermal delivery of medicaments. USA. 4,675,009. Granted Jun. 23, 1987.

[11] HOFFMAN, Allan S. Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*. 2012, **64**(10), 18-23. DOI: 10.1016/j.addr.2012.09.010. ISSN 0169409x. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0169409X12002700

[12] HELLIO, Dominique a Madeleine DJABOUROV. Physically and Chemically Crosslinked Gelatin Gels. *Macromolecular Symposia*. 2006, **241**(1), 23-27. DOI: 10.1002/masy.200650904. ISSN 1022-1360.
Available from: http://doi.wiley.com/10.1002/masy.200650904

[13] HENNINK, W.E. a . Novel crosslinking methods to design hydrogels. *Advanced Drug Delivery Reviews*. 2012, **64**, 223-236. DOI: 10.1016/j.addr.2012.09.009. ISSN 0169409X. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0169409X12002694

[14] BUENO, Vania Blasques, Ricardo BENTINI, Luiz Henrique CATALANI a Denise Freitas Siqueira PETRI. Synthesis and swelling behavior of xanthan-based hydrogels. *Carbohydrate Polymers*. 2013, **92**(2), 1091-1099. DOI: 10.1016/j.carbpol.2012.10.062. ISSN 01448617.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0144861712010909

[15] Hoffman, A. S. Hydrogels for biomedical applications, Adv. Drug Deliv. Rev. 54 (2002),

[16] HOARE, Todd R. a Daniel S. KOHANE. Hydrogels in drug delivery: Progress and challenges. *Polymer*. 2008, **49**(8), 1993-2007. DOI: 10.1016/j.polymer.2008.01.027. ISSN 00323861. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0032386108000487

[17] VAN TOMME, Sophie R., Mies J. VAN STEENBERGEN, Stefaan C. DE SMEDT, Cornelus F. VAN NOSTRUM a Wim E. HENNINK. Self-gelling hydrogels based on

oppositely charged dextran microspheres. *Biomaterials*. 2005, **26**(14), 2129-2135. DOI: 10.1016/j.biomaterials.2004.05.035. ISSN 01429612.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0142961204005678

[18] KAMOUN, Elbadawy A., Xin CHEN, Mohamed S. MOHY ELDIN a El-Refaie S. KENAWY. Crosslinked poly(vinyl alcohol) hydrogels for wound dressing applications: A review of remarkably blended polymers. *Arabian Journal of Chemistry*. 2015, **8**(1), 1-14. DOI: 10.1016/j.arabjc.2014.07.005. ISSN 18785352.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S1878535214001403

[19] RICCIARDI, Rosa, Christine GAILLET, Guylaine DUCOURET, Françoise LAFUMA a Françoise LAUPRÊTRE. Investigation of the relationships between the chain organization and rheological properties of atactic poly(vinyl alcohol) hydrogels. *Polymer*. 2003, **44**(11), 3375-3380. DOI: 10.1016/S0032-3861(03)00246-5. ISSN 00323861. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0032386103002465

[20] BURKERT, Sina, Thomas SCHMIDT, Uwe GOHS, Helmut DORSCHNER a Karl-Friedrich ARNDT. Cross-linking of poly(N-vinyl pyrrolidone) films by electron beam irradiation. *Radiation Physics and Chemistry*. 2007, **76**(8-9), 1324-1328. DOI: 10.1016/j.radphyschem.2007.02.024. ISSN 0969806X.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0969806X07000606

[21] CALÓ, Enrica a Vitaliy V. KHUTORYANSKIY. Biomedical applications of hydrogels: A review of patents and commercial products. *European Polymer Journal*. 2015, **65**, 252-267. DOI: 10.1016/j.eurpolymj.2014.11.024. ISSN 00143057.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0014305714004091

[22] BALAKRISHNAN, Biji, A. JAYAKRISHNAN, E. ALSBERG, K.L. DAMM, K.W. ANDERSON a D.J. MOONEY. Self-cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. *Biomaterials*. 2005, **26**(18), 3941-3951. DOI: 10.1016/j.biomaterials.2004.10.005. ISSN 01429612.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0142961204009329

[23] HAGO, Eltjani-Eltahir a Xinsong LI. Interpenetrating Polymer Network Hydrogels Based on Gelatin and PVA by Biocompatible Approaches: Synthesis and Characterization. *Advances in Materials Science and Engineering*. 2013, **2013**, 1-8. DOI: 10.1155/2013/328763. ISSN 1687-8434.
Available from: http://www.hindawi.com/journals/amse/2013/328763/

[24] ZARZYCKI R, MODRZEJEWSKA Z, NAWROTEK K. Drug release from hydrogel matrices. *Ecological Chemistry and Engineering. S* [online]. Poland, 2010, **17**(2) [cit. 2018-05-25]. ISSN 1898-6196. Available from:

https://www.researchgate.net/publication/228503143_Drug_release_from_hydrogel_matrices

[25] BALAKRISHNAN, B, M MOHANTY, P UMASHANKAR a A JAYAKRISHNAN. Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. *Biomaterials*. 2005, **26**(32), 6335-6342. DOI: 10.1016/j.biomaterials.2005.04.012.
ISSN 01429612. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0142961205003194

[26] SAGHAZADEH, Saghi, Chiara RINOLDI, Maik SCHOT, et al. Drug delivery systems and materials for wound healing applications. *Advanced Drug Delivery Reviews*. 2018, **16**(6), DOI: 10.1016/j.addr.2018.04.008. ISSN 0169409X. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0169409X18300607

[27] ING. NEDOMOVÁ, Eva. *Controlled release of bioactive substances from hydrogel carriers*. Brno, 2017. Doctoral thesis topic. Central European Institute of technology. Supervisor Doc. Ing. Lucy Vojtová, Ph.D.

[28] WANG, Xianfeng, Bin DING a Bingyun LI. Biomimetic electrospun nanofibrous structures for tissue engineering. *Materials Today*. 2013, **16**(6), 229-241. DOI: 10.1016/j.mattod.2013.06.005. ISSN 13697021.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S136970211300196X

[29] KENAWY, El-Refaie, Elbadawy A. KAMOUN, Mohamed S. MOHY ELDIN a Mahmoud A. EL-MELIGY. Physically crosslinked poly(vinyl alcohol)-hydroxyethyl starch blend hydrogel membranes: Synthesis and characterization for biomedical applications. *Arabian Journal of Chemistry*. 2014, **7**(3), 372-380. DOI: 10.1016/j.arabjc.2013.05.026. ISSN 18785352.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S1878535213001603

[30] COVIELLO, Tommasina, Pietro MATRICARDI, Carlotta MARIANECCI a Franco ALHAIQUE. Polysaccharide hydrogels for modified release formulations. Journal of Controlled Release. 2007, 119(1), 5-24. DOI: 10.1016/j.jconrel.2007.01.004. ISSN 01683659.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0168365907000399

[31] PEPPAS, Nikolaos A., Jill E. SCOTT, E. ALSBERG, K.L. DAMM, K.W. ANDERSON a D.J. MOONEY. Controlled release from poly (vinyl alcohol) gels prepared by freezing-thawing processes. *Journal of Controlled Release*. 1992, **18**(2), 95-100. DOI: 10.1016/0168-3659(92)90178-T. ISSN 01683659.
Available from: http://linkinghub.elsevier.com/retrieve/pii/016836599290178T

[32] MURPHY, Diarmaid J., Mayur G. SANKALIA, Ryan G. LOUGHLIN, et al. Physical characterisation and component release of poly(vinyl alcohol)–tetrahydroxyborate hydrogels and their applicability as potential topical drug delivery systems. *International Journal of Pharmaceutics*. 2012, **423**(2), 326-334. DOI: 10.1016/j.ijpharm.2011.11.018. ISSN 03785173. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0378517311010568

[33] KEITA, G., A. RICARD, R. AUDEBERT, E. PEZRON a L. LEIBLER. The poly(vinyl alcohol)-borate system: influence of polyelectrolyte effects on phase diagrams. *Polymer*. 1995, **36**(1), 49-54. DOI: 10.1016/0032-3861(95)90674-Q. ISSN 00323861. Available from: http://linkinghub.elsevier.com/retrieve/pii/003238619590674Q

[34] KUROKAWA, Hidenobu, Mitsuhiro SHIBAYAMA, Takeshi ISHIMARU, Shunji NOMURA a Wel-li WU. Phase behaviour and sol-gel transition of poly(vinyl alcohol)-borate complex in aqueous solution. *Polymer*. 1992, **33**(10), 2182-2188. DOI: 10.1016/0032-3861(92)90886-2. ISSN 00323861. Available from: http://linkinghub.elsevier.com/retrieve/pii/0032386192908862

[35] DAWBER, J. Graham a Stuart I. E. GREEN. An 11B nuclear magnetic resonance study of the reaction of the tetrahydroxyborate ion with polyhydroxy compounds. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases*. 1986, **82**(11), 3407. DOI: 10.1039/f19868203407. ISSN 0300-9599. Available from: http://xlink.rsc.org/?DOI=f19868203407

[36] HUNT, Curtiss D. Dietary Boron is a Physiological Regulator of the Normal Inflammatory Response. *Trace Elements in Man and Animals 10*. Boston: Kluwer Academic Publishers, 2002, , 1071-1076. DOI: 10.1007/0-306-47466-2_327. ISBN 0-306-46378-4. Available from: http://link.springer.com/10.1007/0-306-47466-2_327

[37] SALEHPOUR, Somaieh a Marc A. DUBÉ. Towards the Sustainable Production of Higher-Molecular-Weight Polyglycerol. *Macromolecular Chemistry and Physics*. 2011, **212**(12), 1284-1293. DOI: 10.1002/macp.201100064. ISSN 10221352. Available from: http://doi.wiley.com/10.1002/macp.201100064

[38] HAN, Jingquan, Tingzhou LEI a Qinglin WU. High-water-content mouldable polyvinyl alcohol-borax hydrogels reinforced by well-dispersed cellulose nanoparticles: Dynamic rheological properties and hydrogel formation mechanism. *Carbohydrate Polymers*. 2014, **102**, 306-316. DOI:

10.1016/j.carbpol.2013.11.045. ISSN 01448617. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0144861713011855

[39] YANG, Xiaomin, Kang YANG, Shengwei WU, Xiliang CHEN, Feng YU, Jungang LI, Mingwang MA a Zhiyong ZHU. Cytotoxicity and wound healing properties of PVA/ws-chitosan/glycerol hydrogels made by irradiation followed by freeze–thawing. *Radiation Physics and Chemistry*. 2010, **79**(5), 606-611. DOI: 10.1016/j.radphyschem.2009.12.017. ISSN 0969806X Available from: http://linkinghub.elsevier.com/retrieve/pii/S0969806X09005763

[40] BILANOVIC, Dragoljub, Jeanna STAROSVETSKY, Robert H. ARMON, Shunji NOMURA a Wel-li WU. Cross-linking xanthan and other compounds with glycerol. *Food Hydrocolloids*. 2015, **44**(10), 129-135. DOI: 10.1016/j.foodhyd.2014.09.024. ISSN 0268005X. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0268005X14003300

[41] GHOLAMI, Zahra, Ahmad Zuhairi ABDULLAH a Keat-Teong LEE. Dealing with the surplus of glycerol production from biodiesel industry through catalytic upgrading to polyglycerols and other value-added products: The effects of glycerol and sorbitol. *Renewable and Sustainable Energy Reviews*. 2014, **39**, 327-341. DOI: 10.1016/j.rser.2014.07.092. ISSN 13640321. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1364032114005449

[42] RADHAKRISHNAN, Arun, Gowthamarajan KUPPUSAMY, Veera Venkata Satyanarayana Reddy KARRI, et al. Spray bandage strategy in topical drug delivery. *Journal of Drug Delivery Science and Technology*. 2018, **43**, 113-121. DOI: 10.1016/j.jddst.2017.09.018. ISSN 17732247.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S1773224717306123

[43] GEREMIA, Roberto, Marguerite RINAUDO, Shengwei WU, Xiliang CHEN, Feng YU, Jungang LI, Mingwang MA a Zhiyong ZHU. Biosynthesis, Structure, and Physical Properties of Some Bacterial Polysaccharides. *Polysaccharides*. CRC Press, 2004, 2004-11-30, **79**(5), 606-611. DOI: 10.1201/9781420030822.ch15. ISBN 978-0-8247-5480-8. ISSN 0969806X. Available from: http://www.crcnetbase.com/doi/10.1201/9781420030822.ch15

[44] SINGH, Baljit a Lok PAL. Sterculia crosslinked PVA and PVA-poly(AAm) hydrogel wound dressings for slow drug delivery: Mechanical, mucoadhesive, biocompatible and permeability properties. *Journal of the Mechanical Behavior of Biomedical Materials*. 2012, **9**, 9-21. DOI: 10.1016/j.jmbbm.2012.01.021. ISSN 17516161.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S1751616112000422

[45] JAYARAMUDU, J., G. Siva Mohan REDDY, K. VARAPRASAD, E.R. SADIKU, S. SINHA RAY a A. VARADA RAJULU. Preparation and properties of biodegradable films from Sterculia urens short fiber/cellulose green composites: Mechanical, mucoadhesive, biocompatible and permeability properties. *Carbohydrate Polymers*. 2013, **93**(2), 622-627. DOI: 10.1016/j.carbpol.2013.01.032. ISSN 01448617.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0144861713000489

[46] ALANGE, Vijaykumar V., Ravindra P. BIRAJDAR a Raghavendra V. KULKARNI. Functionally modified polyacrylamide-graft-gum Karaya pH-sensitive spray dried microspheres for colon targeting of an anti-cancer drug. *International Journal of Biological Macromolecules*. 2017, **102**, 829-839. DOI: 10.1016/j.ijbiomac.2017.04.023. ISSN 01418130. Available from: http://linkinghub.elsevier.com/retrieve/pii/S014181301632774X

[47] GARCÍA-ASTRAIN, C., O. GUARESTI, K. GONZÁLEZ, A. SANTAMARIA-ECHART, A. ECEIZA, M.A. CORCUERA a N. GABILONDO. Click gelatin hydrogels: Characterization and drug release behaviour. *Materials Letters*. 2016, **182**, 134-137. DOI: 10.1016/j.matlet.2016.06.115. ISSN 0167577X.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0167577X16310813

[48] MASAYUKI Yamada, SATOSHI Mekata, Aerosol composition containing a film-forming hydroxycarboxylic acid polymer, *Takeda Chemical Industries* 1993 Jun 30. European Patent EU 0 521 455 A2

[49] ARCA, H. Çigdem; SENEL, Sevda. Chitosan based systems for tissue engineering part II: soft tissues. FABAD *Journal of Pharmaceutical Sciences*, 2008, 33.4: 211–216. Available from:
https://www.researchgate.net/publication/228774287_Chitosan_Based_Systems_for_Tissue_Engineering_Part_II_Soft_Tissues

[50] ZHANG, Zhen, Shouyu WANG, Xiliang TIAN, Zexu ZHAO, Jianing ZHANG a Decheng LV. A new effective scaffold to facilitate peripheral nerve regeneration: Chitosan tube coated with maggot homogenate product. *Medical Hypotheses*. 2010, **74**(1), 12-14. DOI: 10.1016/j.mehy.2009.07.053. ISSN 03069877.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0306987709005441

[51] JAYAKUMAR, R., M. PRABAHARAN, S.V. NAIR a H. TAMURA. Novel chitin and chitosan nanofibers in biomedical applications. *Biotechnology Advances*. 2010, **28**(1), 142-150. DOI: 10.1016/j.biotechadv.2009.11.001. ISSN 07349750.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0734975009001852

[52] ARCA, H. Çiğdem, Merve GÜNBEYAZ, Sevda ŞENEL a H. TAMURA. Chitosan-based systems for the delivery of vaccine antigens. *Expert Review of Vaccines*. 2014, **8**(7), 937-953. DOI: 10.1586/erv.09.47. ISSN 1476-0584. Available from: http://www.tandfonline.com/doi/full/10.1586/erv.09.47

[53] SINGH, Baljit, Vikrant SHARMA a Lok PAL. Formation of sterculia polysaccharide networks by gamma rays induced graft copolymerization for biomedical applications. *Carbohydrate Polymers*. 2011, **86**(3), 1371-1380. DOI: 10.1016/j.carbpol.2011.06.041. ISSN 01448617.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S014486171100511X

[54] KIM, Jong Oh, Jung Kil PARK, Jeong Hoon KIM, et al. Development of polyvinyl alcohol–sodium alginate gel-matrix-based wound dressing system containing nitrofurazone. *International Journal of Pharmaceutics*. 2008, **359**(1-2), 79-86. DOI: 10.1016/j.ijpharm.2008.03.021. ISSN 03785173.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0378517308002184

[55] LEVI, Steva, Vladislav RAC, Verica MANOJLOVI, Vesna RAKI, Branko BUGARSKI, Teresa FLOCK, Katarzyna Ewa KRZYCZMONIK a Viktor NEDOVI. Limonene encapsulation in alginate/poly (vinyl alcohol). *Procedia Food Science*. 2011, **1**(3), 1816-1820. DOI: 10.1016/j.profoo.2011.09.266. ISSN 2211601X.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S2211601X11002677

[56] LEE, Kuen Yong, David J. MOONEY, Stefaan C. DE SMEDT, Cornelus F. VAN NOSTRUM a Wim E. HENNINK. Alginate: Properties and biomedical applications. *Progress in Polymer Science*. 2012, **37**(1), 106-126. DOI: 10.1016/j.progpolymsci.2011.06.003. ISSN 00796700.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0079670011000918

[57] MARTINSEN, A., G. SKJÅK-BRAEK, O. SMIDSRØD, E.R. SADIKU, S. SINHA RAY a A. VARADA RAJULU. Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads. *Biotechnology and Bioengineering*. 1989, **33**(1), 79-89. DOI: 10.1002/bit.260330111. ISSN 00063592.
Available from: http://doi.wiley.com/10.1002/bit.260330111

[58] SAYAG, J., S. LIEAUME a S. BOHBOT. Healing properties of calcium alginate dressings. *Journal of Wound Care*. 1996, **5**(8), 357-362. DOI: 10.12968/jowc.1996.5.8.357. ISSN 0969-0700.
Available from: http://www.magonlinelibrary.com/doi/10.12968/jowc.1996.5.8.357

[59] BOUHADIR, K.H., K.Y. LEE, E. ALSBERG, K.L. DAMM, K.W. ANDERSON a D.J. MOONEY. Degradation of Partially Oxidized Alginate and Its Potential Application for Tissue Engineering. *Biotechnology Progress*. 2001, **17**(5), 945-950. DOI: 10.1021/bp010070p. ISSN 8756-7938.
Available from: http://doi.wiley.com/10.1021/bp010070p

[60] BART, Bruce J., Jennifer BIGLOW, J. Corwin VANCE a John L. NEVEAUX. Salicylic acid in Karaya gum patch as a treatment for verruca vulgaris. *Journal of the American Academy of Dermatology*. 1989, **20**(1), 74-76. DOI: 10.1016/S0190-9622(89)70010-4. ISSN 01909622. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0190962289700104

[61] LEUNG, Albert Y. a Steven FOSTER. *Encyclopedia of Common Natural Ingredients Used In Food, Drugs, and Cosmetics*. Second Edition. New York: J. Wiley, 1996. ISBN 0-532-42154-5.

[62] ANDERSON, D. M. W., C. G. A. MCNAB, C. G. ANDERSON, P. M. BROWN a M. A. Pringuer. studies of uronic acid materials, Part 58: Gum exudates from the genus *Sterculia* (gum Karaya). *International Tree Crops Journal*. 1983, **2**(2), 147-154. DOI: 10.1080/01435698.1983.9752749. ISSN 0143-5698.
Available from: http://www.tandfonline.com/doi/abs/10.1080/01435698.1983.9752749

[63] VERBEKEN, D., S. DIERCKX a K. DEWETTINCK. Exudate gums: occurrence, production, and applications. *Applied Microbiology and Biotechnology*. 2003, **63**(1), 10-21. DOI: 10.1007/s00253-003-1354-z. ISSN 0175-7598.
Available from: http://link.springer.com/10.1007/s00253-003-1354-z

[64] GREENE, Theodora W. a Peter G. M. WUTS. *Protective groups in organic synthesis*. 3rd ed. New York: Wiley, c1999. ISBN 978-047-1160-199.

[65] POSTULKOVA, Hana, Ivana CHAMRADOVA, David PAVLINAK, Otakar HUMPA, Josef JANCAR a Lucy VOJTOVA. Study of effects and conditions on the solubility of natural polysaccharide gum Karaya. *Food Hydrocolloids*. 2017, **67**, 148-156. DOI: 10.1016/j.foodhyd.2017.01.011. ISSN 0268005x.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0268005X16304817

[66] SINGH, Baljit, Vikrant SHARMA a Dimpal CHAUHAN. Gastroretentive floating sterculia–alginate beads for use in antiulcer drug delivery. *Chemical Engineering Research and Design*. 2010, **88**(8), 997-1012. DOI: 10.1016/j.cherd.2010.01.017. ISSN 02638762. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0263876210000316

[67] SILVA, D.A, A.C.F BRITO, R.C.M DE PAULA, J.P.A FEITOSA a H.C.B PAULA. Effect of mono and divalent salts on gelation of native, Na and deacetylated Sterculia striata and Sterculia urens polysaccharide gels: note II. *Carbohydrate Polymers*. 2003, 1968, **54**(2), 229-236. DOI: 10.1016/S0144-8617(03)00163-2. ISSN 01448617. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0144861703001632

[68] GOMBOTZ, W, Vikrant SHARMA a Dimpal CHAUHAN. Protein release from alginate matrices. *Advanced Drug Delivery Reviews*. 1998, **31**(3), 267-285. DOI: 10.1016/S0169-409X(97)00124-5. ISSN 0169409X.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0169409X97001245

[69] SINGH, Baljit, Vikrant SHARMA a Dimpal CHAUHAN. Gastroretentive floating sterculia–alginate beads for use in antiulcer drug delivery. *Chemical Engineering Research and Design*. 2010, **88**(8), 997-1012. DOI: 10.1016/j.cherd.2010.01.017. ISSN 02638762. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0263876210000316

[70] MAHDI, Mohammed H., Barbara R. CONWAY a Alan M. SMITH. Development of mucoadhesive sprayable gellan gum fluid gels. *International Journal of Pharmaceutics*. 2015, **488**(1-2), 12-19. DOI: 10.1016/j.ijpharm.2015.04.011. ISSN 03785173. Available from: http://linkinghub.elsevier.com/retrieve/pii/S037851731500318X

[71] LEE, Boon-Beng, Bhesh R. BHANDARI a Tony HOWES. Gelation of an alginate film via spraying of calcium chloride droplets. *Chemical Engineering Science*. 2018, **183**, 1-12. DOI: 10.1016/j.ces.2018.02.049. ISSN 00092509.
Available from: http://linkinghub.elsevier.com/retrieve/pii/S0009250918301118

[72] ANNABI, Nasim, Devyesh RANA, Ehsan SHIRZAEI SANI, Roberto PORTILLO-LARA, Jessie L. GIFFORD, Mohammad M. FARES, Suzanne M. MITHIEUX a Anthony S. WEISS. Engineering a sprayable and elastic hydrogel adhesive with antimicrobial properties for wound healing. Biomaterials. 2017, 139, 229-243. DOI: 10.1016/j.biomaterials.2017.05.011. ISSN 01429612. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0142961217303228

[73] GRIP, Jostein, Rolf Einar ENGSTAD, Ingrid SKJÆVELAND, Nataša ŠKALKO-BASNET and Ann Mari HOLSÆTER. Sprayable Carbopol hydrogel with soluble beta-1,3/1,6-glucan as an active ingredient for wound healing – Development and in-vivo evaluation. European Journal of Pharmaceutical Sciences. 2017, 107, 24-31.

DOI: 10.1016/j.ejps.2017.06.029. ISSN 09280987. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0928098717303731

[74] YOON, Dong Suk, Yunki LEE, Hyun Aae RYU, et al. Cell recruiting chemokine-loaded sprayable gelatin hydrogel dressings for diabetic wound healing. *Acta Biomaterialia*. 2016, **38**, 59-68. DOI: 10.1016/j.actbio.2016.04.030. ISSN 17427061. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1742706116301921

[75] CATANZANO, O., M.C. STRACCIA, A. MIRO, et al. Spray-by-spray in situ cross-linking alginate hydrogels delivering a tea tree oil microemulsion. *European Journal of Pharmaceutical Sciences*. 2015, **66**, 20-28. DOI: 10.1016/j.ejps.2014.09.018. ISSN 09280987. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0928098714003753

[76] CHEN, Bingcan, David Julian MCCLEMENTS a Eric Andrew DECKER. Role of Continuous Phase Anionic Polysaccharides on the Oxidative Stability of Menhaden Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry*. 2010, **58**(6), 3779-3784. DOI: 10.1021/jf9037166. ISSN 0021-8561. Available from: http://pubs.acs.org/doi/abs/10.1021/jf9037166

[77] GARG, Sweta and Ashish GARG. Hydrogel: Classification, Properties, Preparation and Technical Features. Asian Journal of Biomaterial Research. Jabalpur, Asian Journal of Biomaterial Research. 2016, ISSN: 2455-3212.2016, 2(6), 163-170. Available from: https://www.researchgate.net/publication/316075989_Hydrogel_Classification_Properties_Preparation_and_Technical_Features

[78] BARTOVSKÁ, Lidmila and Marie ŠIŠKOVÁ. *Fyzikální chemie povrchů a koloidních soustav*. Vyd. 5., přeprac. Praha: Vysoká škola chemicko-technologická, 2005. ISBN 80-708-0579-X.

[79] BARNES, H.A., J.F. HUTTOM a K. WALTERS. *An introduction to rheology: H A Barnes, J F Hutton, K Walters*. Amsterdam: Elsevier, 1998. ISBN 04-448-7469-0.

[80] Flow properties of polymers: Time- independent fluids. In: *Polymer Properties Database* [online]. CROW, 2015 [cit. 2018-05-17]. Available from: http://polymerdatabase.com/polymer%20physics/images/FluidTypes.png

[81] Flow properties of polymers: Time-independent fluids. *Polymer Properties Database* [online]. Crow, 2015 [cit. 2018-05-17]. Available from: http://polymerdatabase.com/polymer%20physics/Viscosity2.html

[82] MEWIS, Jan and Norman J. WAGNER. Thixotropy. Advances in Colloid and Interface Science. 2009, 147-148, 214-227. DOI: 10.1016/j.cis.2008.09.005. ISSN 00018686. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0001868608001735

[84] Flow properties of polymers: Time- independent fluids. In: *Polymer Properties Database* [online]. CROW, 2015 [cit. 2018-05-17]. Available from: http://polymerdatabase.com/polymer%20physics/images/Time-dependent.png

[85] Formulation Preparation and Characterization Capabilities: Rheometer: TA AR2000 ex. In: *E2P2L* [online]. Solvay [cit. 2018-05-17]. Available from: https://www.e2p2l.com/en/binaries/4.1-Rheometer-175271.png

[86] TA INSTRUMENT. Rheology Theory and Applications Course. 2008. vyd. USA. Available from: http://www.tainstruments.com/applications-library-search/

[87] SPERLING, L. H. Introduction to physical polymer science. 4th ed. Hoboken, N.J.: Wiley, c2006. ISBN 978-0-471-70606-9.

[88] LIU, Changsheng,LIU, Changsheng, Huifang SHAO, Feiyue CHEN and Haiyan ZHENG. Rheological properties of concentrated aqueous injectable calcium phosphate cement slurry. *Biomaterials*. 2006, **27**(2006), 5003-5013. DOI: 10.1016/j.biomaterials.2006.05.043. ISBN 10.1016/j.biomaterials.2006.05.043. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0142961206004947

[89] WYSZECKI, Günter. a W. S. STILES. *Color science: concepts and methods, quantitative data and formulae*. 2nd ed. New York: Wiley, c1982. ISBN 978-0471021063.

[90] ACTON, J.C. a P.L. DAWSON. Impact of proteins on food colour. *Proteins in Food Processing*. Elsevier, 2004, 2004, , 631-668. DOI: 10.1533/9781855738379.3.631. ISBN 9781855737235. Available from: http://linkinghub.elsevier.com/retrieve/pii/B9781855737235500294

[91] Introduction to Colour Spaces. *Graphic Quality Consultancy* [online]. UK: The site wizard, 1997 [cit. 2018-05-24]. Available from: https://www.colourphil.co.uk/lab_lch_colour_space.shtml

[92] The CIELAB color space. In: *Interview helper: Printing technology* [online]. Blogger, 2013 [cit. 2018-05-26]. Dostupné z: http://4.bp.blogspot.com/-ptCTwRd5HYQ/UeiH3gV43rI/AAAAAAAAAQ4/nlftpKrrqYE/s1600/VNMX.png

[93] SOMMER, Lumír. *Základy analytické chemie II*. Brno: VUTIUM, 2000. ISBN 80-214-1742-0

[94] WORKMANJR, J. UV-VIS Spectroscopy Charts. *The Handbook of Organic Compounds*. Elsevier, 2001, 2001, , 63-63. DOI: 10.1016/B978-012763560-6/50010-6. ISBN 978-0-12-763560-6.
Available from: http://linkinghub.elsevier.com/retrieve/pii/B9780127635606500106

[95] NEDOMOVÁ, Eva. *Novel hydrogels based on polysacharides for soft tissue regeneration: Preparation and characterization*. Brno, 2015. Master's thesis. Brno Univeristy of technology. Supervisor Doc. Ing. Lucy Vojtová, Ph.D.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Alg:	Alginate
$CaCl_2$:	Calcium chloride
CIE:	International Commission on Illumination
ECF:	Extracellular fluid
ECM:	Extracellular matrix
EDC:	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
EtOH:	Ethanol
G:	α-l-guluronic acid
GE:	Gelatine
GelMA:	Gelatin methacryloyl
GK(K^+):	Potassium GK salt
Gly:	Glycerol
H_2O_2:	Hydrogen peroxide
HPA:	3-(4-hydroxyphenyl) propionic acid
HRP:	Horseradish peroxidase
M:	β-d-mannuronic acid
MeTro:	Methacryloyl-substituted recombinant human tropoelastin
NHS:	N-hydroxysuccinimide
PVA:	Polyvinyl alcohol
SA:	Sodium alginate
TEA:	Triethanolamine
THB:	Tetrahydroxyborate
VC:	Poly(N-vinylcaprolactam)
UV-VIS:	Ultraviolet–visible spectroscopy

LIST OD APPENDIXES

Appendix 1

Table 1: Measured parameters for CIE lab colour graph

No.	L*	a*	b*	X	Y	Z
31B	74,22	0,15	4,84	44,77	47,05	46,59
31B3	72,98	0,26	5,56	42,99	45,14	43,99
31B5	71,99	-0,93	1,88	41,18	43,65	45,78
31B6	45,53	0,15	7,52	14,21	14,92	13,03
31B7	77,23	0,24	5,43	49,43	51,91	50,99
31B8	12,56	2,76	-4,02	1,52	1,49	2,06
31B9	83,55	-0,70	-2,51	59,78	63,20	71,87
31C6	85,36	0,08	5,04	63,44	66,71	66,53
31C7	15,37	6,15	3,05	2,15	1,98	1,81
31C8	71,6	-2,60	1,01	40,09	43,07	45,93
31C9	55,18	0,00	0,94	21,96	21,10	24,58
31C10	55,04	0,70	4,61	21,98	22,97	22,29
31C11	70,2	0,09	3,73	39,03	41,04	41,40
30E2	90,65	0,06	5,86	73,89	77,71	76,77
30E3	34,71	-0,54	0,08	7,88	8,35	8,86
30E4	71,93	0,27	6,93	41,49	43,56	41,22
30E5	84,87	0,19	5,10	62,58	65,76	65,48
30E6	75,35	0,28	7,83	46,51	48,83	45,63

Appendix 2

Table 2: Counted amount of substances in measured hydrogels

		Mass %			n (mole)		
No.	L*	Borax	PVA	GK	Borax	PVA	GK
31B	74,22	2,22	2,54	0,33	3,61E-04	1,12E-06	2,24E-09
31B3	72,98	2,22	2,54	0,33	3,61E-04	1,12E-06	2,24E-09
31B5	71,99	2,42	2,76	0,18	3,61E-04	1,12E-06	1,12E-09
31B6	45,53	2,66	3,03	0,00	3,61E-04	1,12E-06	0,00E+00
31B7	77,23	2,32	2,65	0,26	3,61E-04	1,12E-06	1,68E-09
31B8	12,56	2,54	2,89	0,09	3,61E-04	1,12E-06	5,61E-10
31B9	83,55	2,14	2,44	0,39	3,61E-04	1,12E-06	2,81E-09
31C6	85,36	2,50	2,85	0,12	5,41E-04	1,68E-06	1,12E-09
31C7	15,37	2,66	3,03	0,00	5,41E-04	1,68E-06	0,00E+00
31C8	71,60	2,63	2,99	0,03	5,41E-04	1,68E-06	2,24E-10
31C9	55,18	2,59	2,96	0,05	5,41E-04	1,68E-06	4,49E-10
31C10	55,04	2,59	2,92	0,08	5,41E-04	1,68E-06	6,73E-10
31C11	70,20	2,53	2,88	0,10	5,41E-04	1,68E-06	8,98E-10
30E2	90,65	3,03	2,30	0,30	5,41E-04	1,12E-06	2,24E-09
30E3	34,71	2,22	2,54	0,33	3,61E-04	1,12E-06	2,24E-09
30E4	71,93	2,75	2,51	0,24	4,51E-04	1,12E-06	2,24E-09
30E5	84,87	1,76	2,67	0,34	2,70E-04	1,12E-06	2,24E-09
30E6	75,35	3,38	2,20	0,28	6,31E-04	1,12E-06	2,24E-09